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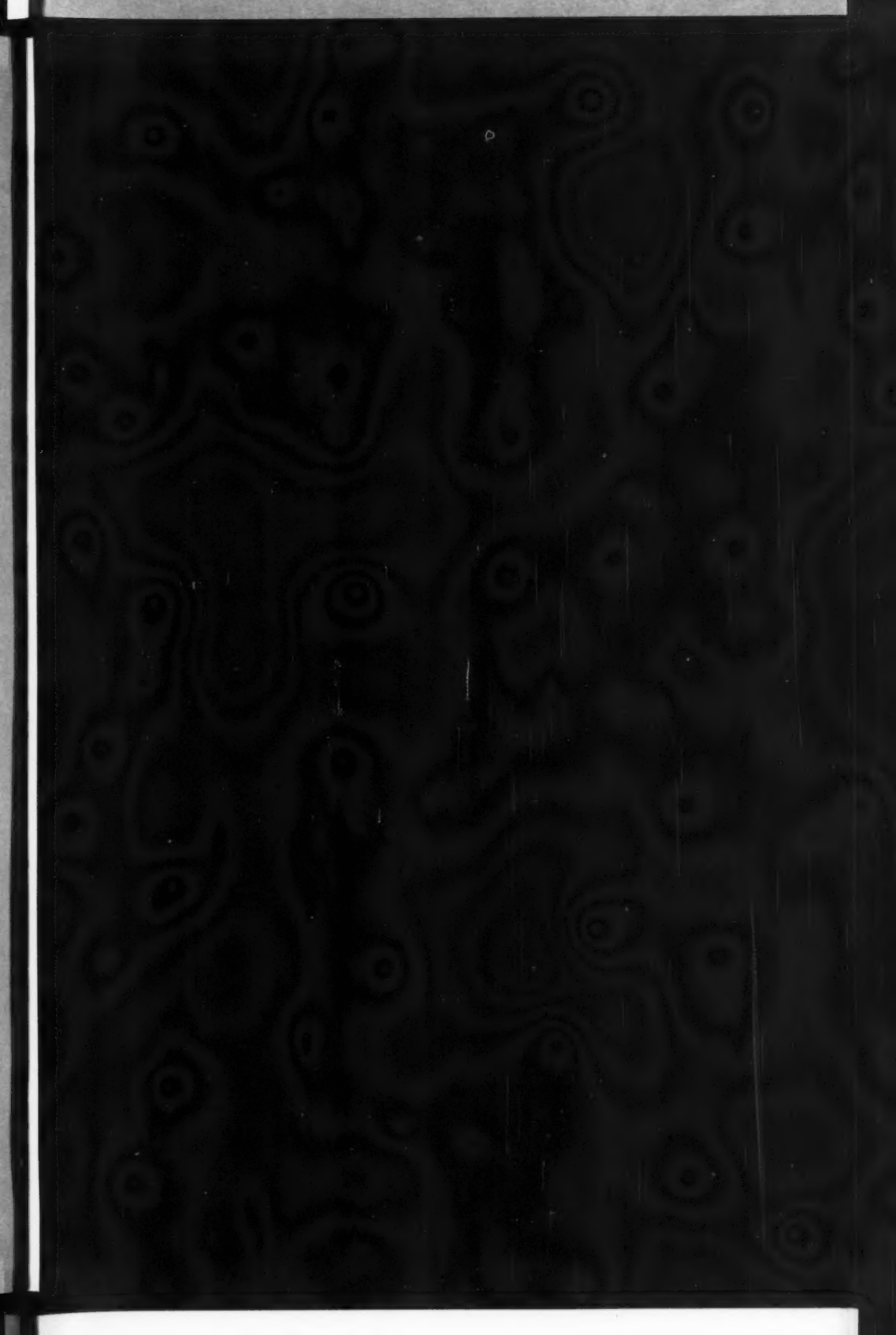

RECENT ADVANCES

IN THE

PHYSIOLOGY OF REPRODUCTION AND HEREDITY

A SYMPOSIUM

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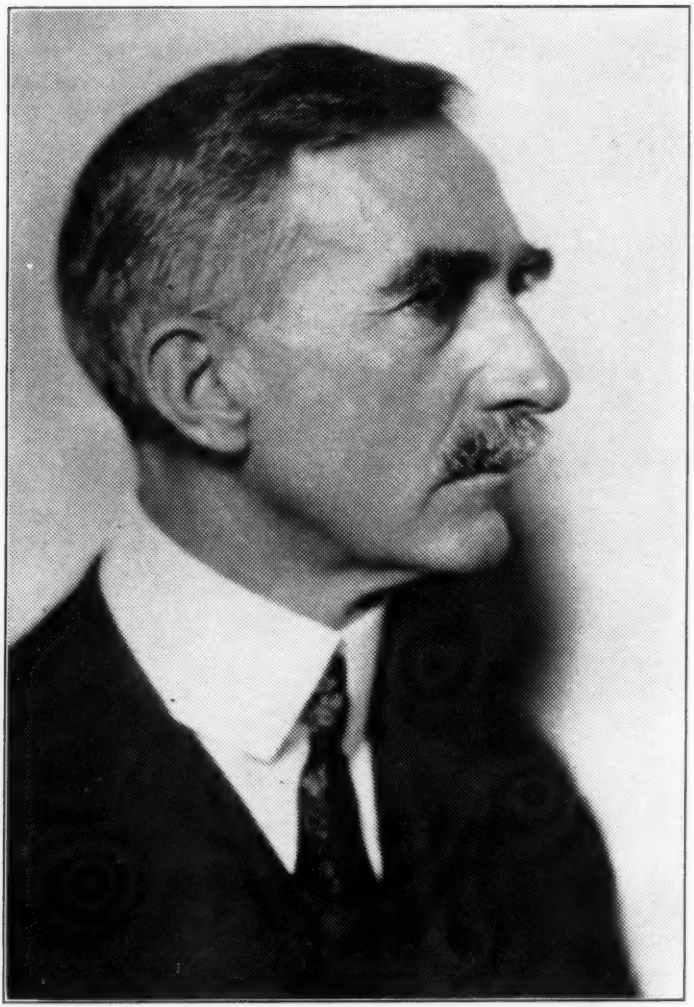

To Frederick Blackmar Mumford, Collegue and Pioneer in the study of Reproduction and Growth at the University of Missouri, this number is affectionately dedicated.

PREFACE

This number contains papers on reproduction and growth presented at a symposium held by the University of Missouri Chapter of the Society of The Sigma Xi on the occasion of the Centenary Celebration (1938-9) of the University of Missouri. The contributors are members, or late members, of the staff of the University of Missouri.

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SOME INVESTIGATIONS OF REPRODUCTION IN PRIMATES*

By

EDGAR ALLEN**

Professor of Anatomy

Yale University School of Medicine

Previous lecturers in this series on "Recent advances in the physiology of reproduction and heredity" have discussed these subjects from their own viewpoints***, determined largely by the living form with which they have worked most. This has included reproduction in both plants and animals. Reproduction has so many varied facets that each different species has something new to offer about the process of reproduction. Dr. Guthrie's work on the bat adds several features not encountered in other mammals. The work on farm animals, presented by Professors Turner and McKenzie has several points of special significance.

For a person working in a medical school there is always present the desire to obtain results which may be applied to the medical or surgical treatment of human beings. Often human beings cannot be used too extensively for experimental purposes, especially where the experiments involve danger to life or health. For that reason during the last fifteen years several American investigators have turned to studies of monkeys and apes for experimental animals. There has been a great deal of research in monkeys which undoubtedly has bearing upon reproduction in human beings. This is especially true of work on internal secretions. Therefore I have chosen to review some of the more recent work derived from the experimental use of monkeys and apes.

As a preliminary, however, a few general statements might be made concerning hormones related to reproduction in lower mammals and certain generalizations which correlate growth and reproduction.

Growth is of great interest to most people. Probably growth of living things gets its first direction from hereditary characteristics.

*This lecture was given before the University of Missouri section of the Society of Sigma Xi on Tuesday, May 16, 1939.

**Formerly, Professor of Anatomy and Dean of the Medical School at the University of Missouri.

***In some ways the present lecture series recalls the 1928 Sigma Xi series, which included lectures by Professors Robbins, Brody, Hogan, Jackson and Greene. This series was published as a book under the title "Growth", edited by Henry D. Hooker, and published by the Yale Press.

Then factors of the environment come into play to make growth possible or to prevent normal growth. Such factors, of course, include climate, food and water, factors of population such as crowding and struggle for survival. They also include factors of the internal environment, hormones circulating in the blood, which are produced in the body by various endocrine glands. We are especially concerned in reproduction with the hormones of the sex glands and the pituitary. There are at least three distinct hormones from the ovaries, as judged by their effects on other reproductive organs. The modern chemist, however, can give us 40 different preparations, chemically pure and of known formula, which have certain specific actions upon the genital organs.

Then apparently there are hormones of the pituitary which act upon the ovaries and make it possible for the ovaries to produce their specific hormones. It is also known that the pituitary glands secrete another distinct hormone which regulates growth of the body. Too much of this hormone will produce giganticism; too little, dwarfism. This brings us logically to the relation between growth of the body and reproduction. In a certain sense reproduction is just a continuation of growth. The infant and later the young boy or girl grows rapidly in body before the maturation of the reproductive organs. Then the growth curve slows down, and reproductive function begins, slowly at first, but then with increasing tempo, until by the attainment of full growth, reproductive function has usually been completely established. One of the limiting factors of continued growth of the body is the fusion of the growing ends of the bones with the shafts, which does not permit further growth in height. After this and other factors limit the increase in size of the individual the resources which previously made this rapid growth possible are diverted to reproductive processes. In most male mammals, with the exception of the seasonally breeding ones, there is more or less continous growth of sperm in the testes,—staggering numbers of sperm being produced in the reproductive lifetime of a single individual. In female mammals, on the other hand, there are cycles of growth and rest in the genital organs. As a generalization it may be said that a spurt of growth in the genital organs raises them to a brief period of function or fruition, if you wish to call it that, during which mating and conception may take place. But if the animal is not mated, there is a regression—a decline of the genital organs and they go into a short period of rest. These recurring cycles, including

growth, function and regression, run like waves through the genital organs in the absence of pregnancy. Probably the outstanding single feature of the whole reproductive history of the female mammal is the period of growth. It is therefore very fitting that a lecture of this sort be submitted for publication in the journal "Growth". In this particular lecture, of course, the interest centers upon growth in the female genital system stimulated by hormones.

There were several deciding factors directing my own interest to research in monkeys. My appointment at the University of Missouri in 1923 followed four post-war years at Washington University School of Medicine. While there, in collaboration with Dr. Doisy and four first year medical students, our early work on ovarian follicular hormone was completed. That was done in mice and rats*. We presented the results in a paper at the Washington University Medical Society and one of the comments from a leading obstetrician was, "These results are all right for mice and rats but they surely do not apply to human beings."

This led to a summer's work in the clinic at Washington University with Dr. Newell, attempting, without much success, to use ovarian hormones in treatment of dysfunction in women. These early hormone preparations were not concentrated enough for successful use in women.

Briefly summarized, what we had found in mice and rats was that females may have sexual cycles without ovulating; therefore the follicles in which the eggs develop must be a source of the hormone regulating the cycles. Previously the corpora lutea, which develop after ovulation, had been considered the most important endocrine tissue of the ovaries. In searching for experimental evidence for an ovarian hormone, fluid from large follicles of pig ovaries was injected into animals after removal of their own ovaries and typical estrous cycles were induced experimentally**. This was the first time that the hormone had been successfully substituted for this endocrine function of the ovaries. But sexual cycles of mice and rats are different from those of women, one difference being that mice and rats do not menstruate.

*References to recent publications are included in the second edition of *Sex and Internal Secretions*, Allen, E., Danforth, C. H. and Doisy, E. A., editors, Williams and Wilkins, Baltimore, 1939.

**A much more detailed review of this subject has recently been published to which the reader is referred for illustrations and references to the literature. See "Sex and Internal Secretions", Allen, Danforth and Doisy (Editors) Williams and Wilkins, Baltimore, 1939.

Therefore, experimental work was begun on monkeys because monkeys are similar to women in many ways—especially as concerns reproductive cycles. My first monkey colony was started at the University of Missouri in the animal house behind the medical school. I remember vividly how the monkeys once escaped into the tree tops in that corner of the campus, and how the whole freshman class of medical students surrounded them, followed the monkeys into those big trees, and chased them back to the cages.

Work with these monkeys led to the first experimental production of menstruation by injections of ovarian hormone.

The last sixteen years have seen completion of much work in monkeys, and some in chimpanzees, which has intensified reinvestigation of the same problems in women, and is now leading to applications of these results to hormonal therapy in women.

Early Work

Because it was done at the University of Missouri and because it has precedence as to time, I intend to describe first some of our early work here with ovarian hormones in monkeys. Then some of the later work will be mentioned in the order in which it occurs in the life of an individual.

The earlier work on mice and rats showed us conclusively that the ovarian follicular hormone was really a *growth hormone,* its growth effects being limited for the most part to the female genital organs. When injected into female animals, it causes a remarkable spurt of growth in the vagina, uterus and mammary glands. It seemed, therefore, that the presence of this hormone was the cause of the growth phase of the sexual cycle, while its decrease or temporary absence was responsible for the phases of regression.

I wish that you might see the various problems that confronted investigators at the beginning of studies in monkeys. One of the first questions was—Do monkeys ovulate during each cycle? Or—Is it possible that they may have menstrual cycles without ovulating? There was some evidence on this point from monkeys collected in the field, and Corner obtained better evidence from close observation of monkeys in his colony at Johns Hopkins Medical School. He showed conclusively that during the summer there may be regular menstrual cycles without ovulation. We found that this was true of monkeys in our colony. To appreciate the importance of this finding you should realize

that at this time the best medical textbooks carried the statement that menstruation occurred because the egg was not fertilized and therefore could not implant in the uterus. The evidence from monkeys clearly showed that eggs need not be produced at all,—consequently there need be no corpus luteum in the ovary,—and still menstruation will occur. As an example I would cite the case of one monkey which had 8 menstrual cycles in one year. When her ovaries were removed and sectioned it was found that she had never produced an egg.

The next question was—When ovulation does occur, at what time of the menstrual cycle does it happen? Also, would it be possible to obtain some monkeys' eggs? Corner had already recovered two, one from the tube and one from the uterus. We recovered several from monkeys in our colony, and always between the onset of two menstrual periods. This practice in finding monkey eggs led later to recovery of the first ripe human eggs in the tubes after ovulation*.

The next problem we wished to study was—What effect would removing the ovaries have on the appearance of the next menstrual period? When the ovaries are removed, for instance, fourteen days after the appearance of the previous menses, the animal begins to menstruate on the eighteenth or nineteenth day instead of on the twenty-eighth as in the normal cycle. This menstrual period following operation is her last menstrual period, for without ovaries menstruation is no longer possible. This latter fact has been known for a long time in women.

Reproductive functions, especially the cyclic sexual phenomena of the uterus, although not entirely independent of the nervous system, can go on quite normally and regularly after all nervous connections to the genital organs have been severed. This has been done by cutting the spinal cord and by cutting the peripheral nerves to the ovaries and the uterus. Although such operative procedures are followed within a few days by menstruation, new cycles begin and continue quite regularly after recovery from the operation.

Another consequence of removing the ovaries from the adult animal was the disappearance of the reddening and swelling of the "sexual skin" which usually faded in ten days or two weeks after operation.

*In collaboration with Dr. J. P. Pratt of Henry Ford Hospital, Detroit, and Dr. Q. U. Newell of Washington University, St. Louis, in the summers of 1928 and 1929.

Then an attempt was made to produce menstruation experimentally, by injecting ovarian hormones into these ovariectomized monkeys. The hormone was obtained by extracting pig ovaries, the extract was dissolved in oil and injected into the monkeys. Injections were made 3 times a day,—morning, late afternoon and midnight,—and continued for two or three weeks. After injections were stopped there was a period of 4 or 5 days and then menstruation began in a majority of the animals. These were the first menstrual cycles to be produced experimentally by injected hormones. The experiment was repeated 4 or 5 times in some animals and took almost a year to complete. Although this experimental menstruation in monkeys was reported in 1927, the first reports of similar work in women were not completed until 1933, when Werner and Collier at St. Louis University, succeeded in inducing menstruation in ovariectomized patients.

After successfully producing menstruation following hormone withdrawal in monkeys, we wanted to study the effect of the hormones during the course of injection. Within 4 or 5 days after beginning injections, the "sexual skin" began to redden and swell and in 10 days or 2 weeks was "full blown" as in the normal animal. There could be no question but that this reddening and swelling of the "sexual skin" was a direct consequence of a certain concentration or threshold of the ovarian follicular hormone. Then studying the vagina and comparing that of the experimental animal with the control, it was shown that many new layers of cells had been added to the vaginal wall. These sections clarified quantitatively the remarkable growth stimulating influence of this hormone. The uterus had also increased greatly in size in the animals injected with follicular hormone. The uterine lining in which the embryo implants may be as much as 6 to 10 times as thick after 3 weeks of stimulation by this hormone as it is in the animals from which the ovaries have been removed for several weeks The glands of the uterus grow into long, straight, tubular structures, similar to their condition in the normal animal at the time of ovulation. The wave of growth which runs through vagina, uterus and mammary glands also affects the uterine tubes. This has recently been shown by experimental work which involves the use of the drug colchicine to arrest cells in mitosis. This shows cell-division in the epithelium of the tube, the new cells apparently replacing old cells which die off

periodically as in most bodily tissues. The mammary glands also grow rapidly in response to this hormone. The ducts of the mammary tree grow extensively and branch and the alveoli bud out from their terminal branches.

As long as the injections of the hormone are continued, growth of these organs continues. Within a few days after injections are stopped growth ceases and these structures retrogress.

Recent Experiments with Estrogenic Hormones

The next experiments were directed toward immature animals, to see if it would be possible to hasten adolescence experimentally. Frankly immature monkeys, weighing about 2 kilograms, injected with this hormone, showed acceleration of growth of the genital organs and precocious attainment of sexually mature conditions.

These results in immature monkeys had a direct bearing on one clinical use of ovarian hormones. Dr. Robert Lewis was very much impressed by the rapid growth of the vagina of the immature animal injected with follicular hormone. He had as patients small girls in an orphanage where chronic gonorrheal vaginitis was a very serious problem. He reasoned that if it would be possible to make the thin, undeveloped vaginal wall of the immature girl grow to a normal adult condition, that it would be better able to combat the infection. After indifferent success in his first trials, his later experiments were successful,—and administration of ovarian hormone has now become an accepted treatment for gonorrheal vaginitis. A month or 6 weeks treatment increases the thickness of the vaginal skin, changes the acidity of the secretions, and usually clears up the infection, except in the most obstinate cases. Gonococci cannot live in acid media. Sometimes there may be a recurrence or a reinfection, but a second treatment is usually successful.

At this time the remarkable effect of the anterior pituitary hormones upon the ovaries was discovered by Smith and Engle and Zondek and Ascheim. Their original work was done in mice, rats and rabbits. Even before extracts of the pituitary were available, we attempted this stimulation of the ovaries by implants of monkey pituitary glands. Five animals on other experiments were being killed at 2 day intervals, and their pituitary glands were implanted in a young female monkey. This treatment stimulated follicular growth in their ovaries, and then

secretion of follicular hormone induced rapid growth in the other genital organs. It was not until several years later that anterior pituitary treatment first successfully produced ovulation in the monkey. Most of the early experiments merely succeeded in producing cystic follicles. But here was another point of interest bearing on clinical conditions, for frequently human ovaries become cystic. Now we have a way to produce experimentally cystic ovaries.

As often happens with a new discovery, investigators began to feel that the pituitary gland was "all important" in stimulating ovarian function. Recently evidence has begun to accumulate that the ovaries react strongly upon the pituitary gland. The following experiment is an example. In a frankly immature monkey 500 rat units of follicular hormone in 10 days are required to produce her first menstrual period. It requires only about 100 rat units in 10 days in an adult monkey. If, now, the immature monkey is injected with 150 units in 10 days, and then the injections stopped, this treatment is not followed by menstruation. If, however, after a short rest, a second series of injections of 150 units is given, the second series, though clearly below the threshold for menstruation in a first attempt, may produce an experimental menstrual period. This points to repetition of sub-threshold doses of follicular hormone as a factor in establishing menstrual rhythm. Since we now know that the ovarian hormone has a profound effect upon the hypophysis, this brings an appreciation of the interaction between ovaries and pituitary, rather than a complete dominance of one by the other.

Swelling of the "sexual skin" has already been mentioned. This is an example of remarkable differentiation of a certain region of the skin. The "sexual skin" is quite different from other skin of the body. If a small patch of it is transplanted to an area on the back or arm it retains its characteristics and reddens and swells at the same time as does that near the genital organs. If a patch of skin from the back or arm is transplanted to the genital region, it fails to react as does the adjacent "sexual skin". Apparently the skin surrounding the external genital organs is differentiated by a mucoid transformation of some of the connective tissue which becomes especially sensitive to ovarian hormone stimulation. This specialized zone develops just before puberty when a raised edge can be seen advancing under the skin, spreading gradually as the animal approaches adolescence.

At the same time the mammary glands begin to grow and spread from the base of the nipple. Up to about the second year of life the sprouts are limited to the region of the nipple, but then about 8 or 10 primary branches grow out, radiating in all directions from the nipple until finally the complete gland spreads over the brest. We now know about how long it takes, and how much hormone is necessary, to grow mammary glands of the immature monkey to full size. For instance, it required 136,000 rat units of hormone and 8 months' time to grow the mammary rudiments of a young male to a size equivalent to those of an adult non-pregnant female. It is easy to so far feminize a male monkey as to grow a pair of typical mammary glands upon him.

In a similar way it is possible to hasten the development of adolescence in young chimpanzees. Not only does their sexual skin "balloon up", and the uterus and mammary glands grow, but the treated females begin to take an interest in males, and be interesting to males. They become receptive to mating after treatment with follicular hormone.

An interesting contribution to our knowledge of adolescence has been forthcoming from studies of the chimpanzee. It is well-known that adolescence in children extends over several years. Development is slow at first, but increases in momentum and culminates in sexual maturity. The order in which these maturing processes occur in the female is extremely interesting. It is perhaps easier to describe them as they occur in the chimpanzee and monkey, since these primates have no restrictions due to morals or customs. A young male and young female chimpanzee were caged together and watched for the development of adolescence. They began sexual relations gradually as a sort of play, just as other non-sexual behavioral reactions develop. The first sign of approaching adolescence in the female was the swelling around the genital organs. This part "ballooned up" for 2 weeks or longer, then decreased and disappeared for 8 or 10 days. Sexual activity varied parallel to these changes. Four or five successive cycles of enlargement and shrinkage occurred at intervals of about 35 days before anything else happened. Then, in between 2 of these periods of swelling, the first menstrual hemorrhage appeared. This particular female had 4 menstrual cycles and then became pregnant. Soon after she became pregnant the genital swelling decreased and disappeared and menstruation ceased. She gave birth to a normal baby and nursed and cared for it during infancy.

This history probably means that follicles developed partly in her ovary, enough to secrete sufficient sex hormone to start a genital swelling, and this happened 4 or 5 times before a high enough hormonal threshold was reached for menstruation. Then 4 menstrual cycles occurred, but, since she did not become pregnant during this time, these cycles were probably without ovulation. She probably became pregnant at the first ovulation. A similar sequence of events in adolescence is found in monkeys and very probably also in girls.

Recently biochemists have recovered and measured the amounts of sex hormones excreted in the urine. While at first investigators were inclined to think that females should excrete only female sex hormones, it is now known that they can excrete some male hormones also, and that males secrete female hormones as well as a preponderance of male hormone. It has also been found that these hormones begin to increase in amount at the time of puberty and that they vary in the amounts excreted during the menstrual cycles and in pregnancy. A normal range has been quite clearly defined, just as normal ranges for growth and body weight at different times during life have been determined. There are now more than 40 different discrete chemical entities which have an action similar to the follicular hormone, and are called estrogens. Whereas the principal source for extracts was ovarian tissue in the early experiment, now it is possible to produce these hormones synthetically and from much cheaper sources.

Before leaving the question of the reaction of young animals to ovarian hormones, it might be well to cite experiments in which young male monkeys were injected with ovarian hormones. Male monkeys, like males of other species, have rudimentary female genital organs. When injected with female hormones these rudiments grow greatly, just as already cited for the mammary glands. The uterus masculinus, for instance, and part of the urethra in the male, react by rapid growth when female hormones are injected. There has been some question as to whether one lobe of the prostate gland reacts similarily to some of these female rudiments in the male, and whether prostatic hypertrophy might be partly due to an increase in the amount of estrogenic hormones in males. At least experimental evidence shows that female rudiments in the male monkey grow when stimulated with female hormones.

Ovulation

Following the recovery of eggs of monkeys from the tubes a definite search was made for human eggs, and in 2 summers' work 5 were recovered,—the first mature eggs to be recovered from woman after ovulation. Human eggs are quite similar in size and appearance to eggs of monkeys and also rabbits. The stage of maturation reached at the time of ovulation in women was found to be the same as in other animals; namely that nucleus of the egg disappears and the chromatin goes into the second maturation spindle at the time the egg is ovulated. staying in this condition unless the egg is fertilized. The time of ovulation in these cases was between the 12th and 15th days after the onset of the previous menses. Later additional human eggs were recovered by Lewis and Pincus, somewhat later in the cycle. Then the problem of time of ovulation in the menstrual cycle was tackled by Hartman in a large colony of monkeys at Johns Hopkins Medical School. He not only studied the ovaries at operation but also perfected the method of rectal palpation whereby, after considerable practice, he was able to tell when the follicle ruptured and the egg was released. This method had the advantage of enabling him to study the time of ovulation in successive cycles in the same animal. He found the mode of ovulation time between the 13th and 14th days of the cycle, confirming with much more extensive evidence the results obtained by earlier investigators.

Studies of the sexual receptivity in monkeys by Ball showed that intercourse was more frequent right about the time of ovulation, and although monkeys would permit intercourse at other times, when the female was allowed to make the choice she was more receptive at the time of ovulation than at other times in the cycle.

The next outstanding achievement in endocrine research was the induction of the ovulation of several eggs at one time in the monkey. This was done by Hisaw, now at Harvard University. He found that after the follicles were stimulated with the *follicle stimulating hormone* from the pituitary, and then the right amount of a second pituitary factor, the *luteinizing hormone* added, that ovulation of as many as 5 eggs at once could be induced. This carried further the work previously described by Smith and Engle in which they induced ovulation of 50 or more eggs at one time in the rat. These experiments

make us wonder if at some previous time ancestral primates were not litter-bearing animals, and perhaps the reduction in the amount of pituitary hormone acting upon the ovaries might have reduced the number of eggs produced. They also provide one reason for multiple birth.

Other methods of determining the time of ovulation are: (1) the recognition of corpora lutea in ruptured ovarian follicles, (2) the discovery of the sharp rise in electrical potential when measured between the cervix and the symphysis pubis when follicles rupture, (3) recognition of an end product of progesterone which can be recovered from the urine, and (4) mating at a single restricted period of each cycle to see whether or not pregnancy follows. Much data acquired for ovulation time in woman shows that more frequently ovulation comes at the mid-point between the onset of 2 successive menstrual periods, although occasionally it may occur at other times in the cycle.

Further Studies of Menstruation and Cyclic Changes in the Uterus

One of the greatest subjects of controversy among students of the human uterus has been the extent to which the surface is removed at menstruation. Sometimes a uterus will be found from which the surface has been completely denuded. Others are found in which slight bleeding occurs without much damage to the inner surface. Still others are found in which a part of the lining may be intact and parts denuded. This has made it difficullt to generalize about the condition of the menstruating uterus. Studies of the monkey have been very important because it is possible to take samples of the uterine surface at will, both of the normal uterus and of the uterus after various experimental procedures.

The recent study by Daron has added much to our knowledge of the blood vessels of the uterus and the mechanism of menstrual bleeding. The principal vessels enter the uterus at the sides in the broad ligaments. They pass through the muscle layers and then send branches into the lining of the uterus. These branches are of two sorts, short straight branches which supply only the deeper third of the endometrium, and long coiled branches which extend all the way to the surface. Apparently before menstruation begins the coiled vessels are constricted and the flow of blood either slowed or stopped. This reduces the supply

of blood to the outer portions of the lining of the uterus and causes serious damage to this tissue. Then these coiled arteries open and as the blood flows back into the vessels again, some of it leaks through the walls and forms small puddles and lakes in the surrounding tissue. This blood then escapes into the lumen of the uterus and appears as menstrual blood.

Our conceptions as to the actual mechanism of menstruation have been derived from how we thought it should happen from studying the uterus after it had been fixed and cut in sections. Recently Markee has perfected a method whereby he can actually watch menstruation in uterine tissue transplanted to the eye. Small bits of the lining of the uterus are inserted into the anterior chamber of the eye and there they attach to the iris and can be watched as they go through the same cyclic changes as in the uterus. The monkeys with the transplants are held in position without anesthesia while the observer watches this tissue through a microscope.

When there is plenty of ovarian hormone circulating in the blood, the transplants are pink and the vessels can be seen pulsating rhythmically. If the ovaries are removed from the monkey, the uterine transplant blanches, the vessels constrict, and the whole transplant shrinks. Then if follicular hormone is injected the tissue blushes again. As long as hormone is injected, this tissue blushes and grows. Soon after the hormone is stopped, the transplants begin to decrease in size and bleed—menstruate. As the pieces of transplant shrink, the coiled arteries close up like springs, and this increased coiling retards the circulation of the blood, bringing it practically to a standstill. This stasis of blood may last for as long as 24 hours before menstrual bleeding begins. Soon after this vasoconstriction becomes acute, leucocytes begin to wander through the vessels out into the tissues. This is a clear sign that the tissues supplied by these vessels have been injured by lack of oxygen and other necessary supplies. The constriction of these coiled arteries lasts for a long time, to a certain extent throughout menstruation, except when individual arteries relax to permit circulation of the blood for brief periods. When this happens, the blood escapes through the wall into the surrounding tissue and enters the anterior chamber of the eye just as it enters the cavity of the uterus. After a short period of a few minutes of bleeding these individual arteries may

contract again and so control the hemorrhage. There seems to be no synchronization of action of these vessels, one opens, bleeds, then contracts, to be followed by others in other parts of the uterus.

In the course of this bleeding, there is a crumpling of the tissue and fragments are lifted free and sloughed off from the surface. Remember that while this is occurring in the superficial part of the uterine wall, the deep part is being supplied continuously by blood from the straight vessels. After a period of 3 or 4 days of this intermittent bleeding, growth sets in very rapidly, and within sometimes a few hours the surface of the endometrium denuded at that menstrual period is completely repaired. The rapidity of this healing is truly amazing. It has been watched in the transplant in the monkey's eye and seen to occur in certain cases in 2 hours time.

One important point settled by these actual observations of menstruation in transplants in the monkey's eye is that there is very little difference in the process when an egg is successfully ovulated and a corpus luteum formed, and when the egg fails to ovulate and the follicle regresses. As far as the vascular changes and the fundamental processes in this tissue are concerned, no corpus luteum hormone is necessary.

Menstrual cycles of any desired length can be produced at will by extending the period of injections of estrogenic hormones or by following the estrogenic hormones with progesterone.

There is considerable evidence now to show even during the latter half of ovulatory cycles when corpora lutea are functional in the ovaries, some estrogen is still secreted by the ovaries. It has been found that pure preparations of progesterone alone are not nearly so effective in producing the progestational changes as when small amounts of estrogen are added. It is very probable that these 2 hormones act together and that progesterone represents a transformation of the basic molecule of estrogen.

Before leaving the question of menstruating bits of transplanted uterus in the monkey's eye, it is worth mentioning that when pieces are transplanted in both eyes and stimulated by hormone administered systemically, and then a crystal of follicular hormone is implanted in one eye, this crystal keeps the endometrium of that eye growing after injections of hormone are stopped, while the transplant in the other eye

retrogresses and menstruates. This is a local action of the crystal planted close by the tissue in one eye.

It is also worth mentioning in this connection that if the follicular hormone in the blood is maintained at a sufficiently high level, menstruation can be prevented entirely. For instance, Zuckerman has injected a monkey every day for a year, and the uterus remained in a growing condition and did not menstruate.

Synergistic Action of Hormones

From the evidence cited to date we could conclude that a single hormone is responsible for menstruation. This hormone produces the type of lining of the uterus which occurs between 2 mentrual periods. Withdrawal or decrease of hormone is followed by menstruation.

However, the uterus stimulated by follicular hormone alone is not capable of supporting the life of an implanted embryo. In order to permit pregnancy additional changes are required and these are brought about by a hormone secreted by the corpus luteum which develops in the ruptured follicle if ovulation occurs. This second ovarian hormone, progesterone, has also been extracted from ovarian tissue, crystalized and recently synthesized. Although it has been possible to produce in monkeys the changes in the uterus which permit implantation, it has not been possible to carry the monkeys through complete pregnancy with this hormone. Corner and Willard Allen have, however, succeeded in doing this in the rabbit. The rabbits were mated, the eggs fertilized and allowed to pass down into the uterus. Then the ovaries containing the recent corpora lutea were removed and their endocrine function replaced by injected progesterone. The embryos implanted normally and mothers gave birth to normal litters of young at the usual time.

The monkeys have, however, served as experimental animals in some interesting experiments with progesterone reported by Hisaw. He found that: (1) if the monkey was injected first with follicular hormone, then (2) with the hormone of the corpus luteum, and then (3) the uterus traumatized by being opened to take out a small piece of tissue, that the lining of the uterus would develop the same condition that it does in the normal animal when the embryo arrives in the uterus for implantation. Three factors are needed: first, stimulation with the follicular hormone; second, stimulation with the corpus luteum hor-

mone; and, third, the trauma of taking a biopsy specimen. This procedure calls forth the growth of epithelial cells of the surface of the uterus and these cells grow down into the connective tissue just as a tumor from the skin grows into the subcutaneous tissue. In extreme cases this new growth may spread all over the inside of the uterus. This is a condition typical of the implantation site in the monkey. It has no homologue in man.

Another example of synergistic action between 2 hormones is the control of formation and secretion of glycogen by the uterine epithelium. The glands of the surface epithelium of the uterus form and store glycogen in reaction to the follicular hormone. Globules of glycogen can be seen between the nuclei of the cells and the basal membrane. These cells do not secrete the glycogen until stimulated by progesterone, then the glycogen passes through the cell and is extruded from the free end.

Beside this specific action on the cells of the lining of the uterus, progesterone in some way prevents the onset of menstruation. This can be done experimentally. If a monkey is injected for 10 days with estrogen and then this followed by injections of progesterone, as long as the progesterone is injected, menstruation will not appear. The impending menstrual period can be held off for several weeks or even months. In this way the action of progesterone is similar to the action of estrin, and appears to prevent the degenerative phase of the cycle. It has since been shown that the male hormone, testosterone, has a similar effect in holding off the menstrual period which usually follows cessation of treatment with estrogenic hormones.

During pregnancy there is a profound change in the balance of internal secretions in the body. Very soon after implantation of the embryo there is a distinct rise in the anterior pituitary hormone, which is excreted in large amounts in the urine. Recognition of this is used as one test of pregnancy. As pregnancy progresses the amount of this hormone decreases markedly. The amount of the estrogenic hormone, on the other hand, rises to a peak toward the end of pregnancy. For a considerable period we were at a loss to know why this estrogenic hormone should increase during pregnancy because the ovaries are probably at a low plane of function. Then it was discovered that the placenta, which develops to make the connection between mother and

embryo, contains large amounts of estrogenic material, and as the placenta grows in size with the advance of gestation the amount of this hormone in the circulation increases. It was then shown in women that the ovaries could be removed toward the end of the second month of pregnancy without upsetting this increased amount of hormone in the blood. This showed very definitely that it was the placenta rather than the ovaries which was secreting estrogenic substance during pregnancy.

Just before birth there is a considerable growth of the uterus and mammary glands, the latter in both male and female fetuses. Within a few days after birth female babies may menstruate, and from both male and female babies there is sometimes a slight amount of milk-like fluid which can be expressed from the nipples. These two things follow the reduction of estrogenic hormone in the circulation. There can be little doubt that normally the high concentration of estrogenic hormone in the mother's blood may pass through the placenta and affect the genital organs of the fetuses. Fortunately, however, this influence does not become strong until the genital organs have already been molded, either in a male or female direction, and consequently it causes little modification of their genetic constitution. Now that purified and concentrated hormones are available for experiments, it has been demonstrated by injecting these into the mother, that they do pass through the placenta and affect the fetuses. For instance, mother rats injected with male sex hormones gave birth to litters apparently made up entirely of males. Upon more careful study, however, it was found that half of the young were females but failure of proper formation of the lower part of the vagina left it without an opening to the outside. There was also a failure of development of the nipples. Male mice and rats do not have nipples. In other words, there was masculinization of genetic females by the male hormone injected into the mother. Although this type of experiment is much more clear-cut in rodents some striking changes have been obtained in monkeys.

Toward the end of pregnancy there are changes in the bony pelvis of monkeys. They are not as pronounced as in the pocket gopher or in the guinea pig, but still there is a relaxation of some of the joints of the pelvis, and as in the lower animals these changes are probably controlled by successive action of 2 or more hormones.

Growth of the mammary glands in response to estrogenic hormones

has already been described briefly. Some animals require the successive action of estrogenic and luteal hormones for complete growth of the mammary glands. In monkeys, apparently, the first hormone is more important. Recent work of Dr. Turner's indicates that ovarian hormones may stimulate the pituitary to secrete a mammary growth hormone. After the glands have been grown under the action of these ovarian hormones, it is possible to induce them to lactate by injecting the hormone, prolactin, from the anterior pituitary gland. This causes the alveoli and ducts of the glands to fill up with milk-like secretion. Attempts are being made to use prolactin clinically in women, but if the mammary gland is not first fully grown, the effects of the last hormone are not very successful. Failure of any one of several hormones may prevent successful lactation.

Aging, Menopause and Post-menopausal Changes

One extremely interesting aspect of this question has been the rapid disappearance of hormones from the blood stream soon after injection. In attempting to find where they are destroyed in the body, investigators have circulated them through combinations of organs by perfusion with salt solution or serum instead of blood. Estrogenic hormones perfused through a combination heart, lungs, and kidneys last for quite a while. If, however, a liver is inserted into this series of organs, the hormone is destroyed rapidly. It seems that the liver is the principal organ providing resistance against too high a concentration of hormones, as it seems to be against toxic agents of other sorts in the circulation.

So much experimental evidence has accumulated to explain the growth changes which occur in response to floods of hormonal stimulation that we are now in a position to explain, to a certain extent, the decline of these processes during old age, and the most likely explanation appears to be a failure of some of these growth-producing hormones. The crucial question in connection with the female genital organs has been—What endocrine gland begins to decline first, the ovaries or the pituitary? We have seen that both are essential for changes in the uterus.

We have known for a long time that as a woman ages the number of eggs in her ovaries is greatly decreased. In fact, one thing which always impresses the medical student in studying sections of the ovaries

is the scarcity of eggs in the human ovary as old age advances. While it is possible to stimulate ovarian function in aging animals somewhat by injecting anterior pituitary hormones, the available evidence leaves the impression that the ovary loses its power to function as an endrocrine organ. In old age the ovaries shrink to such a small size that it is very difficult to find anything but small masses of connective tissue. The uterus also becomes very small and atrophic. The mammary glands wither and decrease in size. Decline of ovarian secretion is probably the primary cause for the menopause. That it is not a decline in the secretion of the pituitary hormones which affects the ovaries is shown by the increase in those substances excreted by aging persons past the menopause. This brings us to the question—Will it be possible to replace these ovarian hormones in aging people. There seems no logical reason why we should not strive to replace the sex hormones, just as we do the hormone of the thyroid gland in cretins, or the hormone of the pancreatic islands in diabetics. In fact, therapy at and after the menopause is an increasing use for this hormone. In cases of severe menopause symptoms, where the transition is difficult, ovarian hormones are prescribed. It would seem best at present, however, to administer these hormones in tapering doses, merely aiming to "tide over" a patient until the endrocrine balance again becomes established. We still have too little evidence to be sure that use of these hormones in aging people is not dangerous. Besides, what woman of 60, after her reproductive life is past, wishes to menstruate again? If a level of hormone treatment below the threshold for menstruation could be established as beneficial in alleviating menopause symptoms, it should be useful therapeutically.

Sometimes in old age chronic degenerative diseases affect the genital organs and are very difficult to treat successfully. Short periods of growth stimulation by ovarian hormones assist greatly in alleviating these conditions, but they may recur after the hormone is stopped.

There are other dangers in the use of these growth stimulating hormones in aging people. It has been shown quite conclusively that long continued treatment with these growth stimulating substances is followed by tumors and cancers of genital organs in experimental animals. It is true that a genetic factor of susceptibility or resistance to this type of growth is also involved and some animals do not respond by tumor

formation. The incidence of cervical and mammary cancer rises rapidly at menopause and in aging people. More than 20% of all cancer is cancer of the female genital organs. The indescriminate use of growth stimulating hormones may be a serious factor in starting flare-ups of local growths in these organs, which during normal reproductive life have been subject to flood and ebb tides of growth stimulating hormones. At least until we are sure, it would be safer and wiser not to use these growth stimulants widely in aging people.

It is obviously difficult to attempt to summarize a lecture of this sort. Perhaps the best that can be done in this direction is to emphasize the most important things briefly. In my opinion the most important single feature is that estrogenic hormone from the ovary acts as a powerful growth stimulant to the female genital organs, and this hormone is the primary factor in the mechanism of recurrent sexual cycles. Its secretion is apparently dependent upon the gonadotropic secretion from the anterior pituitary, but in return ovarian hormones react to regulate the secretion of the pituitary gland. Furthermore, this powerful growth stimulant, acting continuously without periods for rest, may stimulate abnormal growths in the uterus and mammary glands, which, if genetic factors for cancer susceptibility are present, may be the deciding factor in the growth of uterine or mammary tumors and cancers. Therefore these hormones should not be used too extensively in concentrated form until the limits of their action are better understood from animal experimentation.

GROWTH AND REPRODUCTION AS PROBLEMS IN THE SYNTHESIS OF PROTEIN MOLECULES

By

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The last previous lecture in this symposium furnished us with a study of chromosomes and of the behavior of genes. In that lecture Dr. L. J. Stadler expounded the viewpoints of modern geneticists about the nature of the string of genes which we know must exist in each chromosome. The topic for our present consideration is an extension of the same line of thought.

Genes are understood to be minute units of matter, complex in structure, but each with a definite size and construction, so that each has a very definite degree of potency. Their material must be protein, and may very likely be nucleo-protein. They have two outstanding characteristics, in that (1) they can bring about the formation of new gene particles, exact duplicates of themselves; and (2) they can catalyze the formation of special substances that influence the happenings in the rest of the cell. The first of these characterizations indicates that they are conspicuously similar to materials now known as virus proteins; the second of these characterizations, that they conform to the description of substances which we know as enzymes. A quick and ready account of the major traits shown by the genes, then, would be to say that they are substances which are at once viruses and enzymes, although possessing, to be sure, some special traits of their own.

Both viruses and enzymes are proteins and both viruses and enzymes have been separated successfully in pure crystalline form by research workers in very recent years. Thus it has become a very simple and logical step to infer that single genes are single molecules comparable to the molecules of these crystalizable substances.

One of the most interesting lines of discovery in the last five years has been the development of our knowledge of viruses. If there is any one thing that might be described as the "missing link" between living and non-living, corresponding on the one hand to ordinary chemical crystalline material and on the other hand showing the traits associated with life, it is viruses, of which the outstanding example is the tobacco mosaic virus. This has now more than once been crystalized as slender

white needles with a satiny sheen. Chemically these crystals consist of a nucleo-protein, and as a pure chemical material they apparently can be kept indefinitely, not undergoing any of the distinctive processes of life. If dissolved in the sap of a living tobacco plant cell the molecules of this virus show power of reproduction through the assimilation of materials present in the sap. Thus every molecule is equivalent to a parasitic organism feeding upon the tobacco plant. The size of the particles changes with the conditions of solution, but one of the most stable conditions is when the protein, including its combined nucleic acid, shows a molecular weight of 18,000,000. This is equivalent to

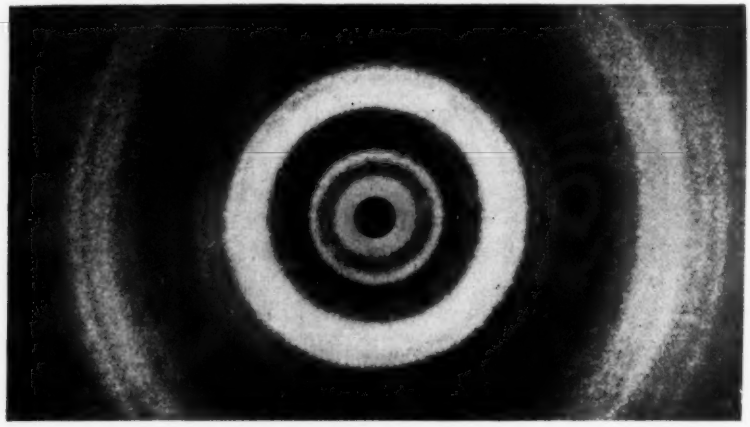

Fig. 1. Diffraction picture of tobacco virus protein. (Wyckoff and Corey).

about five hundred times the weight of an albumin molecule, and is of the order of about one ten-thousandth the mass of the smaller forms of bacteria.

In line with modern procedures in the study of molecular structures Wyckoff and Corey have obtained X-ray diffraction pictures from powdered samples of these crystals. Fig. 1 represents a positive from such an X-ray negative. Each pale circle here represents a specific grid-spacing in the lattice work of the molecule, or in the crystal that is being exposed. The circles farthest out on the periphery give witness to the finer structures. Those circles nearest the center are projected by lattice crevices of larger dimensions. Unfortunately, quite a number of the rings undoubtedly represent a sort of overtone

TABLE I (From Wycoff and Corey)
PRINCIPAL X-RAY DIFFRACTION LINES OF POWDERED CRYSTALLINE TOBACCO MOSAIC PROTEINS.

Ordinary strain		Aucuba strain		Ultraviolet irradiated protein
Spacing	Intensity	Spacing	Intensity	Spacing
Å.		Å.		Å.
80	Strong	80	Strong	
53	Medium	55	Medium	
37	Faint	37	Faint	
28	Very faint	27	Very faint	
20.8	Medium	21.0	Medium	21.2
16.2	Very faint	14.2	Very faint	14.7
14.2	Very faint		Very faint	
11.0	Strong	11.0	Strong	11.3
10.2	Faint	10.2	Faint	
9.2	Medium	9.2	Medium	9.2
7.44	Medium	7.43	Medium	7.5
6.5	Faint	6.5	Faint	
5.7	Very faint			
5.44	Faint	5.41	Faint	
4.95	Medium	4.95	Medium	
4.71	Medium	4.68	Medium	
4.44	Medium	4.45	Medium	4.46
4.08	Faint	4.09	Faint	4.10
3.88	Medium	3.88	Medium	3.88
3.70	Very faint			
3.54	Very faint			
3.39	Faint	3.39	Faint	

echo of other larger, more fundamental spaces. This makes the actual geometrical interpretation of a picture such as we have here extremely difficult. The first step in the interpretation is, however, given on Table I. It will be seen at once that the diffraction rings indicate a very wide range of lattice spaces and presumably a very complex molecule and crystal. The largest spacings listed undoubtedly represent crystal rhythms given by spacings between molecules. Many of the smaller spacings must certainly be ascribed to rhythms in the arrangement of atoms within the molecule. We may call attention to the strong band representing eleven Ångstrøm units, as it is very characteristic of a long list of proteins to have this strong lattice spacing at about 10 to 11 units.

The bands representing the lower end of the scale are reporting atom lattices only slightly larger than the standard distances between atoms

in an organic compound, as 1.5 Ångstrøm units is a reasonable approximation for the average inter-atomic space in the bonds of carbon to carbon, carbon to oxygen, and carbon to nitrogen.

In order to understand the space problems of protein molecules, which are the crucial molecules in the structure of living material, it is necessary to start with a consideration of the orientation of carbons in the amino-acids. The carbon most crucial in determining the orientation of parts in amino-acids is the one known as the "alpha carbon", lying between the carboxyl carbon and the amino group. In the physiologically significant amino-acids this alpha carbon invariably has the orientation designated as "levo". The four amino-acid diagrams of

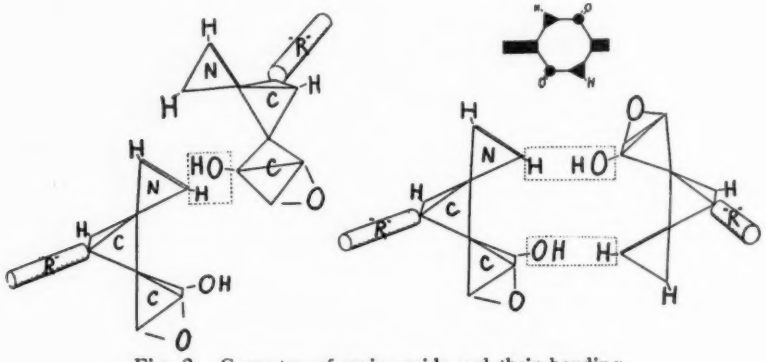

Fig. 2. Geometry of amino-acids and their bonding.

Fig. 2 represent the alpha carbon in the "L" position. The purpose of this figure is to show two different major ways in which amino-acids having the biologically standard configuration may approach each other and react with each other. If two amino-acids come together as indicated on the left hand half of the figure, loss of the equivalent of one water molecule between them can unite them and there results a serpentine which can be continued in a serpentine style indefinitely. The non-active portion of the amino-acids in this serpentine project alternately on opposite sides.

The two amino-acids represented on the right hand half of Fig. 2 are shown brought together in such a way as to split off two molecules of water and produce a ring compound or hexagonal pattern. The non-reacting residues of amino-acids brought together in this pattern

project approximately in the same direction. As here illustrated, they are projecting forward from this paper. At first glance this pattern appears to be a closed system, unsuitable for building into larger complexes. This is not, however, correct, as we see from the next figure.

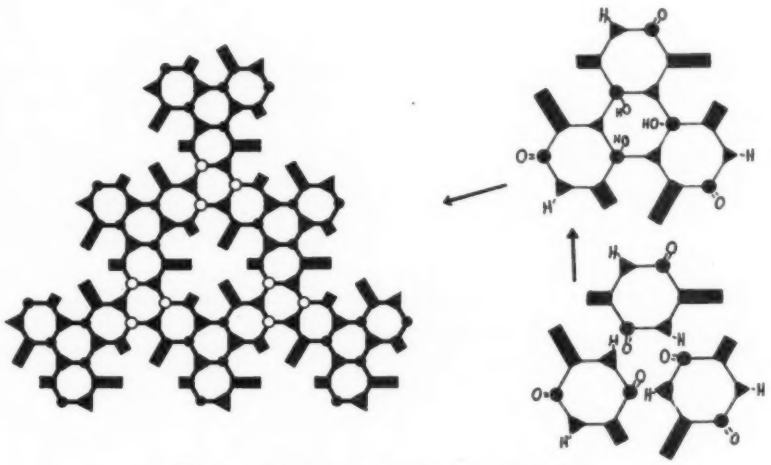

Fig. 3. Structure of supposed cyclol lamellae.

This diagram (Fig. 3) shows a way in which such pairs of amino-acids can come together into triangular groups of six amino-acids each. This figure is drawn in terms of the symbolic code indicated in Fig. 2. The process is one which need not halt at this point, but can go on practically indefinitely, producing as the next step triangles with 18 or 36 amino-acids, or hexagons with 24. After this the pattern can expand into large sheets, or "mono-layers", as they are called, in the form of almost perfectly flat surfaces. We have thus the geometrical basis for the hypothesis that the "mono-layers", or lamellae of the proteins have this hexagonal structure, which is called by Wrinch the "cyclol" pattern. It conforms nicely to Langmuir's account of protein mono-layers that are hydrophilic on one face by virtue of hydroxyl groups, and hydrophobic on the other face by virtue of alkyl groupings. In other respects the cyclol pattern does not develop any great polarity. A complete molecule would in this case consist of several mono-layer units, probably duplicating each other, and spaced presumably about 10 or 11 Ångstrøm units apart. We illustrate an imaginary development (Fig. 4) of this pattern into a molecule containing 4×142 amino-acids.

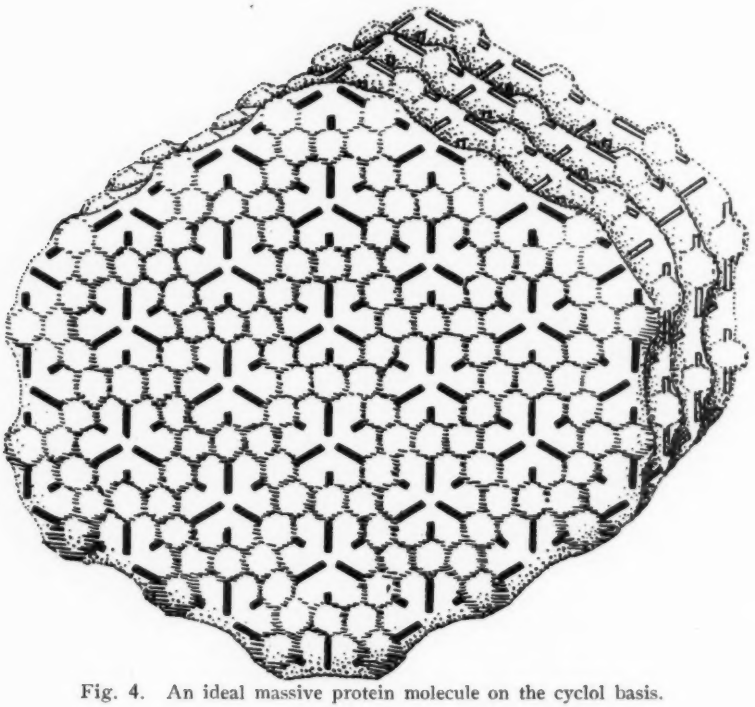

Fig. 4. An ideal massive protein molecule on the cyclol basis.

Going back to the serpentine manner of linkage between amino-acids we find protein structures that have actually been demonstrated in some of the natural proteins (Fig. 5). We see here an approximate representation in diagram of clupein, a protamine (basic protein) occurring in the nuclei of the ripe spermatozoa of the herring. In Fig. 6 is a diagram of a possible extension of this linear pattern to become a broad sheet or mono-layer. The horizontal zig-zag in this pattern corresponds to the zig-zag in the preceding pattern. Vertical cross-connecting lines represent bonds formed after the same manner as we saw in the cyclol

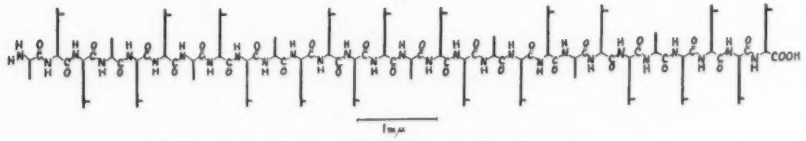

Fig. 5. Polypeptide chain, as exemplified by a protamine.
(Diagram constructed after Linderstrom-Lang)

pattern, but this time making a distinctly different type of grid. The amino-acid residues and the reactive hydroxyls of this pattern each emerge on both sides of the plane of the lattice. There is experimental evidence that the proteins of hair and horn may take on approximately the lattice structure here indicated.

The types of protein lattice already indicated have received the most attention in the recent literature of the subject, but they by no means cover all the suggestions that have been made, still less exhaust the set of geometrical possibilities. One form proposed for special cases is a blend of the longitudinal fiber form with that of the six-membered ring (Fig. 7). Other geometrically plausible modes of union of amino-acids include further variants of lamellar mono-layers either with the "R" groups and hydroxyl groups segregated on opposite sides of the lattice, producing respectively a hydrophobic and a hydrophilic face of

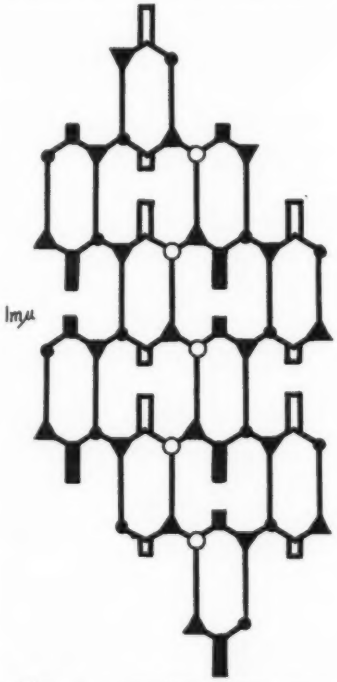

Fig. 6. Possible lattice of a fibrous protein lamella. (Diagram constructed after Astbury and Woods).

Fig. 7. Amino-acid chain, partly extended keratin form. (Diagram constructed after Astbury and Woods).

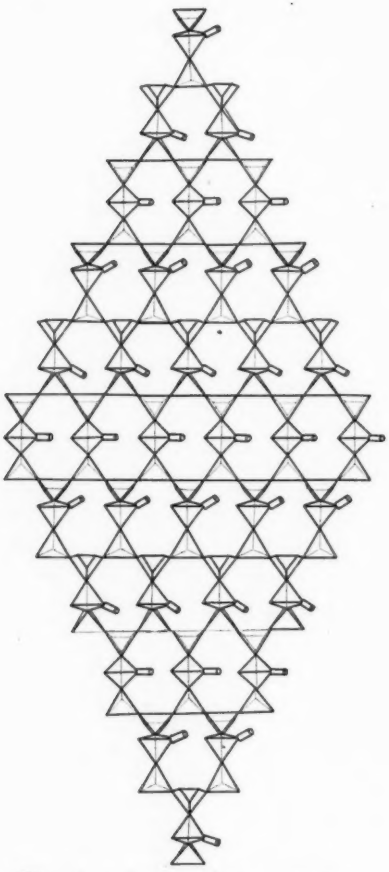

Fig. 8. An imaginary mono-layer having hydrophilic and hydrophobic faces.

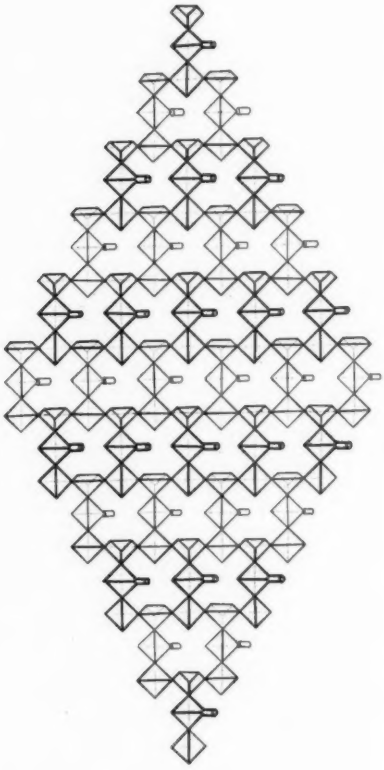

Fig. 9. An imaginary mono-layer having no distinction of hydrophilic or hydrophobic faces.

the mono-layer, (e. g. Fig. 8) or with both groups occupying both faces (e. g. Fig. 9). Spirals and large rings having rather definite dimensions can also fit into the conditions set by the geometry of these amino-acid groupings. This is especially true for configurations laid out on the basis of 5-atom cyclic bondings (Fig. 10).

The size of protein molecules is by no means a random matter. Svedberg, working at the University of Upsala, has shown that nearly all water-soluble proteins have molecular weights that come within a certain very definite frame-work. That frame-work appears to represent

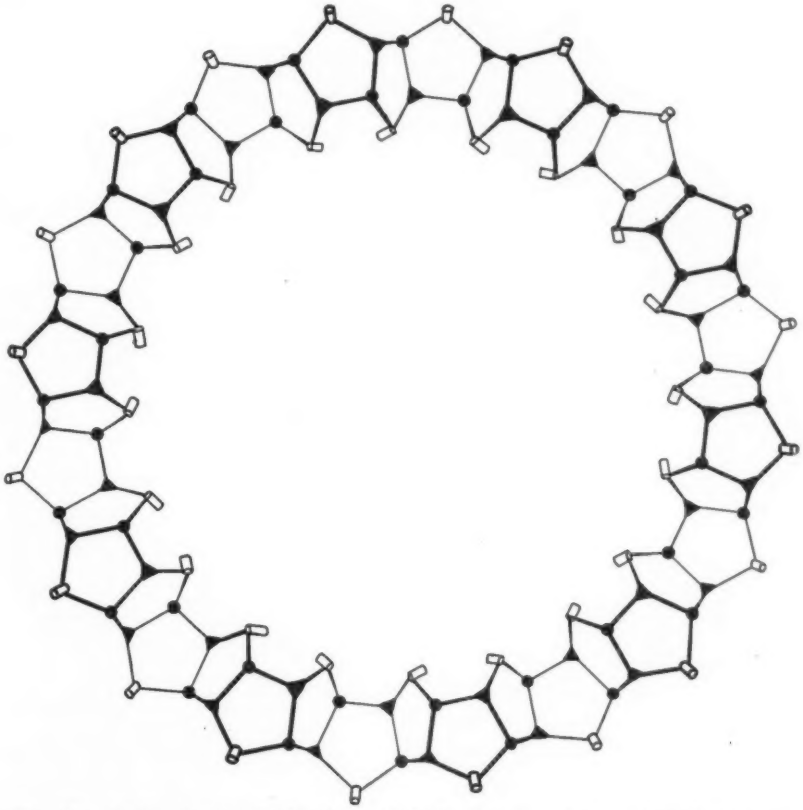

Fig. 10. An imaginary coil of amino-acids built of 5-atom rings, and producing an open circle structure.

integer multiples of a primary molecular weight approximating 35,000 to 36,000 (Table II). Nearly all proteins seem to have either this molecular weight or this weight divided by 2 or multiplied by the numbers, 2, 4, 8, 12, 24, 48, 96, etc. Such a relationship implies a definite size and shape for the unit lamella in the protein molecule. The Svedberg unit, averaging 35,200, corresponds to the weight of a polypeptide having about 288 amino-acid groups of average size in each molecule. In the case of the cyclol pattern it might easily be supposed that a unit lamella might be a triangle or hexagon of some definite size;—for example, possibly 36 or 72 amino-acids to a lamella. Fig. 11 gives us a representation of possible ways in which such triangular lamellae, each

carrying 36 units, might be brought together into closed molecules of definite size. The figures here shown consist entirely of triangular or rhombic (double triangular) faces. Hexagons are not useable because polyhedra cannot be formed out of them. The forms we see are the regular tetrahedron, or equilateral triangular pyramid; the triangular hexahedon, in shape equivalent to 2 tetrahedra pressed against each other, the hexahedron with rhombic faces; and the regular octahedron.

TABLE II
REPRESENTATIVE MOLECULAR WEIGHTS OF PROTEINS
(ABBREVIATED FROM SVEDBERG)

	Molecular weight by sedimentation speed	Calculation of Svedberg units
Lactalbumin Myoglobin	17,500 17,200	1 X 17,600 = 17,600
Gliadin	26,000	
Zein Lactoglobulin Pepsin Insulin	35,000 41,800 35,500 40,900	2 X 17,600 or 1 X 35,200 = 35,200
CO-hemoglobin (man) Serum albumin (horse)	63,000 70,200	4 X 17,600 or 2 X 35,200 = 70,400
Canavalin	113,000	6 X 17,600 or 3 X 35,200 = 105,600
Phycocyan Serum globulin (horse)	131,000 167,000	8 X 17,600 or 4 X 35,200 = 140,800
Antipneumococcus serum globulin (man)	195,000	
Phycocyanin Edestin Amandin	272,000 309,000 329,000	16 X 17,600 or 8 X 35,200 = 281,600
Hemocyanin (Palinurus) Urease	446,000 483,000	24 X 17,600 or 12 X 35,200 = 422,400
Thyroglobulin (pig)	628,000	
Hemocyanin (Nephrops) Hemocyanin (Homarus)	820,000 752,000	48 X 17,600 or 24 X 35,200 = 844,800
Erythrocruorin (Planorbis)	1,636,000	96 X 17,600 or 48 X 35,200 = 1,689,600
Hemocyanin (Octopus)	2,785,000	
Hemocyanin (Rossia) Erythrocruorin (Lumbricus)	3,316,000 3,140,000	192 X 17,600 or 96 X 35,200 = 3,379,200
Hemocyanin (Helix)	6,630,000	384 X 17,600 or 192 X 35,200 = 6,758,400

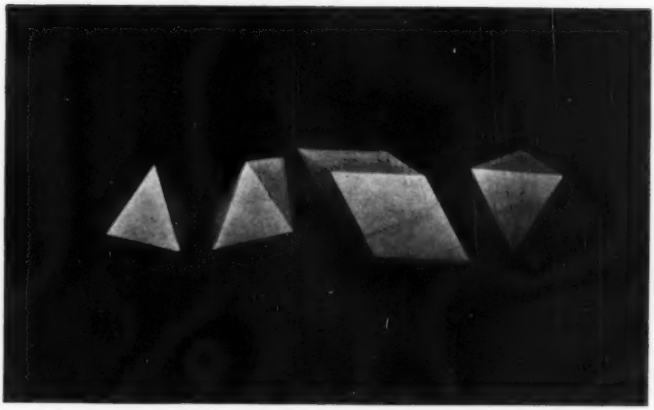

Fig. 11. Polyhedra built of triangular or rhombic faces.

It has been proposed that the regular octahedron (Fig. 12) carrying 36 amino-acids on each side, or 288 amino-acids in all, might well be

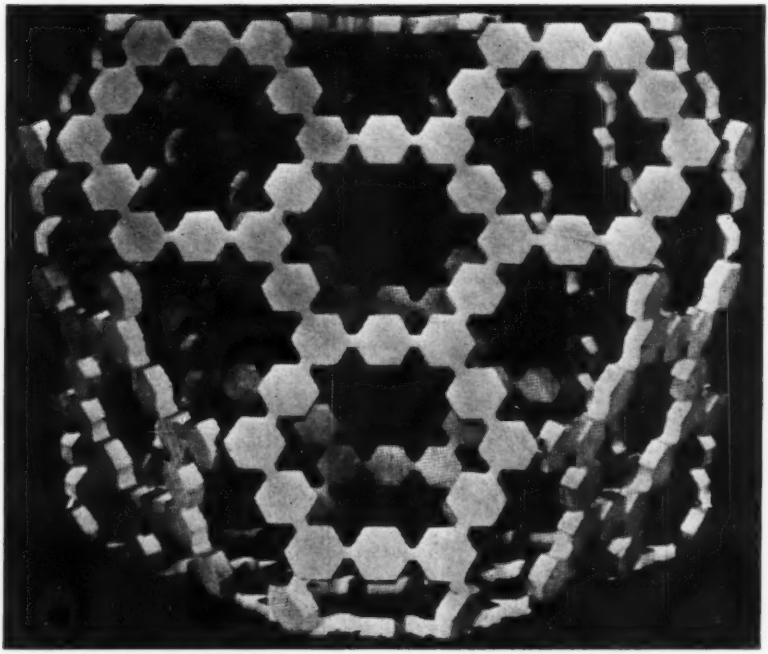

Fig. 12. Octohedron model as suggested by Wrinch for insulin protein. (Proc. Roy. Soc. **A.161.**

the foundation unit of this Svedberg series of molecular weights. We have here the reproduction of an approximately octahedral figure, diagramed in such a manner that each small hexagon represents a cyclol group of 2 amino-acids. Wrinch has proposed, on the basis of a certain amount of evidence, that insulin and probably various other proteins, have molecules of this type. In this connection an X-ray diffraction pattern from an insulin crystal was obtained by Crowfoot. Application of the Patterson-Fourier mathematical procedure led to a series of diagrams which represent the product of the electron densities at points separated by a constant vector. One of these is shown in Fig. 13.

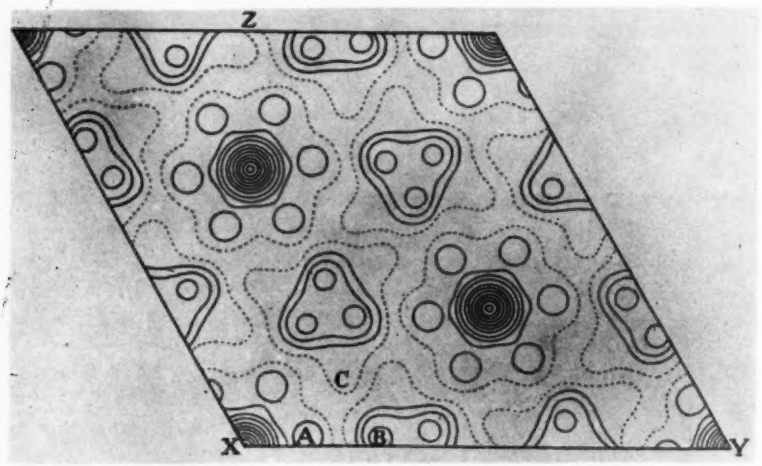

Fig. 13. Patterson-Fourier crystal analysis of insulin electron field. (Crowfoot)

Combined results from these diagrams agree with an arrangement of peaks at the six angles of an octahedron. But the agreement does not extend to the relative intensities of the peaks or to the finer structure of some of the diagrams, and there has resulted a great difference of opinions as to its closer interpretation. Both Crowfoot and Bernal doubt whether these fields fit any specific pattern yet proposed. Wrinch and Langmuir feel that with one or two supplementary postulates they fit the Wrinch hypothesis remarkably well. Other authors protest that they could be interpreted to fit more than one pattern.

We must proceed now to consider a little further how these various geometrical relationships enter into the problems of protein synthesis

and gene reproduction. One may realize by now that any pattern that can be ascribed to a protein molecule must be decidedly comparable in many respects to that of a very minute crystal, as it will inevitably contain just such a rhythm of its constituent parts as does a crystal. Correspondingly, the electrical forces characteristic of a crystal lattice should show themselves within such a molecule and upon its surface. Now a crystal has a very distinctive power of growth by pulling new atoms or atom groups into appropriate positions in its own pattern. The electrical fields that are responsible for the growth of the crystal are effective within a range of a few Ångstrøm units only. If we suppose, then, that the surface of a protein is laid out in such a manner that dissolved amino-acids have access to it, it is to be inferred that the forces which it is known can successfully produce crystals will in a similar manner draw the amino-acid into appropriate positions upon the lamellar surface of a giant protein molecule. This activity, carried on to completion, means nothing more nor less than the reduplication of the mono-layers out of which protein molecules are constructed. Remembering the close parallellism between protein molecules and the crystal structures of less complex materials, we have here an apparently adequate mechanism for a self-reduplicating force in protein molecules. Saying the same thing in other words, a molecule having such a power may be looked upon as an enzyme capable of catalyzing the production of more of itself. Exactly this is what we see in the case of the viruses, and exactly this is what we must in all reason anticipate in the case of the genes—the power of self-reduplication through a catalytic influence upon the arrangement of available amino-acids. For this synthesis the mono-layers already present obviously must serve as patterns that determine a corresponding arrangement of amino-acids in the new mono-layers that take form upon them. Doubtless the catalytic potency of many proteins may be at too low a level to produce results without the assistance of an additional enzyme, but in the case of viruses and of genes, the molecule which provides the mono-layer pattern is itself sufficiently potent as a catalyst, and may therefore be described as a molecule capable of reproductive activity.

As stated just above, it is only possible that such a power should work within range of a very few Ångstrom units. A protein built in layer-cake style out of parallel mono-layers could very well lay on additional mono-layers one after another, until the molecule reached double the

original size, when it might be drawn apart into two molecules to fit more perfectly into the crystalline pattern. But is it possible to suppose that proteins in the form of closed polyhedra can do this same thing? Let us look at the two polyhedral forms that are most available and see how they relate to this problem (Fig. 14). On the left is a cluster of tetrahedra. We see at once that if they should form new lamellae upon the faces of the old lamellae the results would not be tetrahedra, or at least not until heavy re-arrangement had taken place. The regular tetrahedron is a figure that permits neither of close packing, nor of packing in such a manner that the interspaces are also tetrahedral in shape. In order to have polyhedra that can carry out a smooth auto-

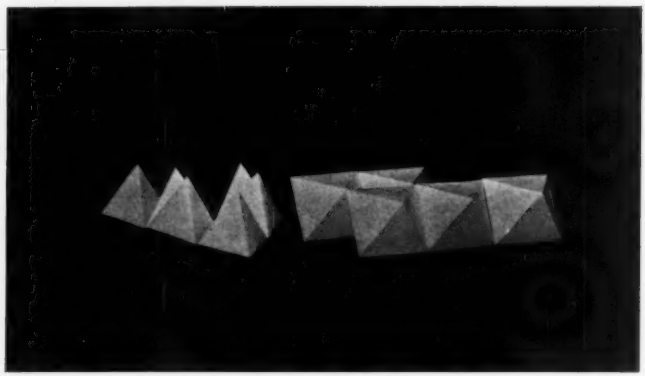

Fig. 14. Tetrahedra and octohedra, showing their inability to pack closely.

synthesis, it is necessary that they should be able to range their molecules in a crystal lattice having interspaces the same shape as the molecules bounding them. Upon the right hand side of the figure we have a similar demonstration for octahedra. They, too, are incapable of being packed in such a way that the formation of new lamellae would constitute the synthesis of new octahedra. I incline to believe, therefore, for geometrical reasons, that substances with high auto-synthetic powers can hardly be tetrahedra or octahedra.

A much more favorable case is the hexahedron build of rhombic faces. Figure 15, left half, might be imagined as equivalent to a system of cubes that have been pulled out until their 90° angles have been transformed into angles of 60 and 120 degrees. Like cubes they will pack solidly. Also like cubes, if packed in an open rhythm,

omitting every second hexahedron in the lattice, a reduplication of the mono-layers of the hexahedra still remaining would constitute the completion of a close-packed stack of hexahedra.

We see on the right hand side of Fig. 15 that a similar building up of the spaces for a lattice of octahedra would generate regular tetrahedra. It might be conceived that in a large moist crystal aggregation of octahedra it would be possible to form as a first step tetrahedra, equivalent to halves of octahedra, and then by shuffling the parts of two tetrahedra an ultimate development of a new octahedron might come

Fig. 15. Modes of packing possible in hexahedra and octohedra.

about. Such a process represents syntheses that might conceivably occur in a matrix of numerous molecules, but it could not occur through the auto-synthetic activity of a single unique molecule.

Let us turn now more specifically to the problem of the chromosome and the gene. The accumulating evidence points to the likelihood that the gene is a single giant molecule, or a joint or a node in what we might call a super-giant molecule. It has power of self-reduplication. The arguments we have just given lead one to turn away from any suggestion that a gene is a closed polyhedron. The chief remaining alternative is that its molecule is of layer-cake pattern, such, for example, as was shown in Fig. 4 or such as might be constructed from other monolayer types (figs. 6, 8, 9, and the like). We come back, therefore, to the lamellar type of protein and the supposition that a single gene comprises a single huge molecule of this type. The possibilities included are;—(1) the chance that it may be, in a technical sense, a simple protein, not conjugated with any non-protein accessory material;

or (2) it may be a nucleoprotein, or, (3) it is possible that some other prosthetic group or groups may be present, either along side of nucleic acid or without any direct inclusion of nucleic acid.

The chromosomes, or the chromatin of which chromosomes are

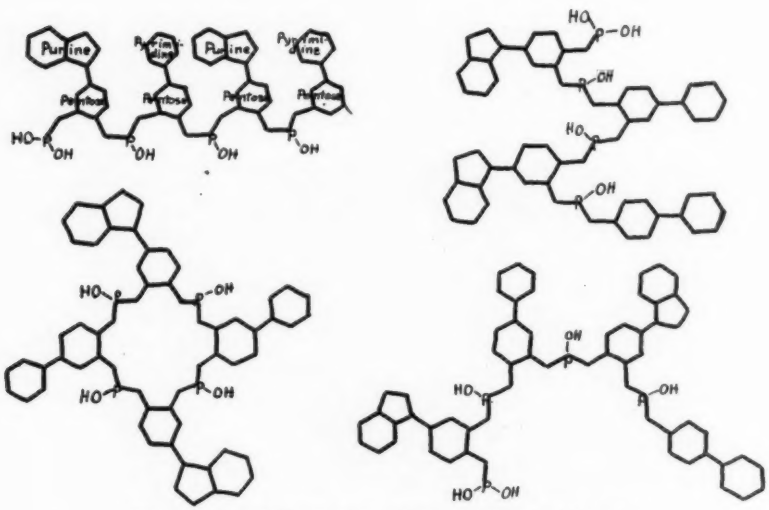

Fig. 16. Possible configurations of thymonucleic acid.

constructed, are definitely nucleo-proteins and this substance if not necessarily a part of the actual gene, it is at least a part of its most immediate environment, not to say mother substance. Furthermore, the tobacca mosaic virus, with its very gene-like attributes, is definitely a nucleo-protein. So let us consider what goes into the structure of nucleo-proteins. Nucleic acid, such as occurs in the nuclei of the vertebrates, is composed of the materials indicated in Figure 16, and its arrangement of parts in space must approximate one of the four alternatives shown in this figure. It may possibly be further complicated by aggregation of those units into particles of colloidal size. Its acidity is derived from the replaceable hydrogens on the remaining hydroxyls of the phosphoric radicles. In the chromatin this nucleic acid is united as a salt with a very basic protein. There is much possible variety in the protein, but in at least some instances this protein has the linear polypeptide structure of the protamines (Fig. 5). This gives us the three important ingredients in a chromosome; namely,

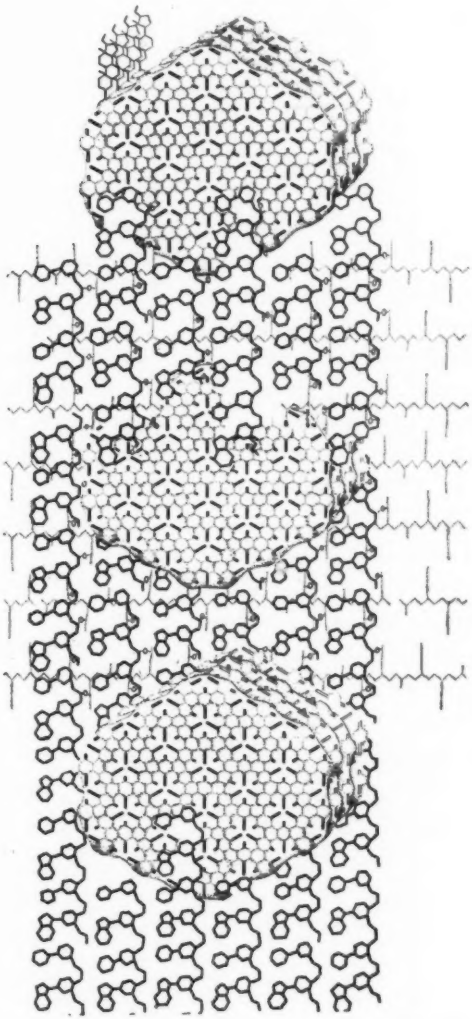

Fig. 17. Ideal reconstruction of chromatin, with genes.

genes, nucleic acid, and basic protein, the latter two in a salt combination with each other.

Indulging our imaginations in an attempt to bring the three constituents of the chromosomes into reasonable chemical and topographical relation to each other, our conclusions point to something after the

manner of Figure 17. Three giant protein molecules of layer-cake type serve to indicate how the genes may lie distributed in a single elo..gated string. As a bit of fantasy we have attached to one of these molecules 4 units of a prosthetic group having a sterol configuration. The chromatin matrix in which these genes lie contains approximately equivalent reaction weights of basic fibers, indicated transversely, and nucleic acids with the enlongated dimension represented as running lengthwise of the chromosome. We choose this arrangement for the basic protein and nucleic acid because the nucleic acid is the ingredient which carries the same electrical charge at both ends. The basic protein, on the other hand, is known to carry at least sometimes a carboxyl at one end and an amino group at the other, so that its molecule possesses two opposite poles. Accordingly, if in these cases the longitudinal fibers of the fabric were composed of the protein it would be impossible to reverse the orientation in a segment of a string of genes. As we know that this reversed orientation is experimentally possible, it is more likely that the longitudinal binding effect is derived from nucleic acid and not from basic protein*. Such a picture as we have here includes, of course, many completely arbitrary details. Yet it seems reasonable to suppose that the more fundamental facts in the actual arrangement of gene and chromosome constituents may approximate themselves more or less to the sketch suggestion that is here presented.

As was mentioned before, there is a considerable likelihood that the gene molecule is a conjugated protein, consisting of protein in a salt-like union with nucleic acid. Some, at least, of the viruses have this composition, which corresponds also to the structure of chromatin. Furthermore, the points at which the genes are located in the giant salivary chromosomes of fruit flies are known to be especially rich in nucleic acid.

We know too little about the combinations of nucleic acid with proteins of the large-moleculed type to attempt to make a diagram, but we can be confident that whatever the mode of union between these two

*Since this article went to press, a study by Mazia and Jaeger (Proc. Nat. Acad. Sci., U. S. A., Vol. **25**: 456-461; see also Proc. Internat. Genetics Congr. 1939) has shown that in the case of the nuclei of insect salivary glands the chromosomes still retain their identity and shape after all nucleic acid has been digested away. Our conceptions of chromosome structure must be revised accordingly, to allow purely protein substances to contribute to the framework of these chromosomes in all three dimensions.

may be, the crucial protein material in the gene must keep to a lamellated form.

In line with this general concept of the structure of genes and of the nature of the process of gene synthesis, the question what happens in the mutation of a gene may well be reviewed and reinterpreted. A mutation is reproduced true to form from the very first. It must, therefore, be an alteration located upon the protein lamella—or in a prosthetic group merged into this lamella—on the actual pattern which sets the form for all subsequent syntheses. We observe that it alters the course of events in the rest of the cell. It constitutes, therefore, some sort of a modification in the catalytic machinery of the gene, so that there is a change in the nature or effectiveness of the active principle (or principles) that the gene emits into the rest of the cell. Such a change may be minute, but must always be a definite, discontinuous alteration of a chemical entity, namely the lamelliform, auto-catalytic, enzymatic protein of the gene.

Not all physiological syntheses need be strictly auto-syntheses. Many cell constituents and many secreted proteins must be very weak in their auto-synthetic capacity, and their production may actually be more dependent on a distinctive enzyme produced independently of the molecules that serve as patterns. It is even possible that some specialized cell products owe their constitution entirely to special enzymes, and not at all to pattern molecules. We may well imagine that keratin. (the protein of hair) derives its constitution in this way. Certainly one would hesitate to claim that the germ cell contains pattern molecules for keratin, for white connective tissue and for all like substances.

Thus our survey has made us acquainted with several concepts that are applicable to the fundamental processes of life, in a manner that contracts into much narrowed dimensions the gulf that has seemed so unbridgeable between things living and things non-living.

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REPRODUCTIVE CYCLES IN ANIMALS WITH SPECIAL REFERENCE TO THE BAT

By

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The continuity of species depends upon reproduction, or the origin of new individuals, which occurs by either the asexual or sexual method. In the latter, two germ cells which may be conspicuously differentiated as egg and spermatozoön in two specialized individuals unite to form a single cell. These germ cells are not produced continuously even by sexually mature animals, especially in the case of the female. There is a reproductive cycle which is distinguished, essentially, by a recurrence of the presence of mature germ cells.

Species differences in the reproductive cycle directed attention to the fact that its control was obviously related to the integration of the whole organism. Methods of study of the factors conditioning the coordination of this bodily function have usually been two, the study of wild animals collected at intervals throughout the year and the observation of normal and experimental laboratory animals. From these studies it is known that some species have only one reproductive cycle a year while others have more than one. Many wild animals, such as fishes, frogs, reptiles, and some birds and mammals, such as the fox, have only one reproductive cycle a year in the temperate zone. Other birds and mammals will breed several times during a certain part of the year—hedgehogs litter twice, the vole four to five times each season (Deanesley, '34, and Brambell and Rowlands, '36). Modification of the normal wild-type cycle may occur under conditions of captivity or domestication. The wild rabbit usually has a limited breeding season but may have two litters a year, while the domesticated varieties have cycles throughout the year. The domestic fowl produces eggs for the greater part of the year and some wild birds will continue to lay eggs if brooding is prevented by their removal. Four- to five-day estrous cycles recur throughout the year in the absence of pregnancy in the laboratory rat and mouse. When pregnancy occurs, cycles will begin immediately after parturition, if the young are removed, or after lactation and continue until pregnancy or senescence intervenes.

Such a range of variation suggests that numerous factors condition the successive stages of the reproductive cycle and their timing (Fig.

1). What part does heredity play? For certainly the complete analysis of any biological problem cannot neglect the genes and their activities. Little can as yet be stated about specific genes related to reproductive cycles. That the type of germ cell an individual produces can be referred to its genic make-up is probable. It seems certain that the number of germ cells formed is determined by specific genes in Drosophila. Failure to mature gametes is a consequence of a single mutation in mice. Allen ('37) has suggested that variations in lengths of reproductive cycles are conditioned by heritable differences in rate of cell growth and specialization. Heredity does play a part but the data are insufficient to determine just what and study should be confined to material in which not too many hereditary variables are present. Such material can be studied with reference to its environment.

It has long been known that reproductive cycles under natural conditions are strikingly related to the seasons. Many species breed as the days grow warmer and longer (frogs and birds) and some germ cells mature as the days grow colder and shorter (sheep and deer).

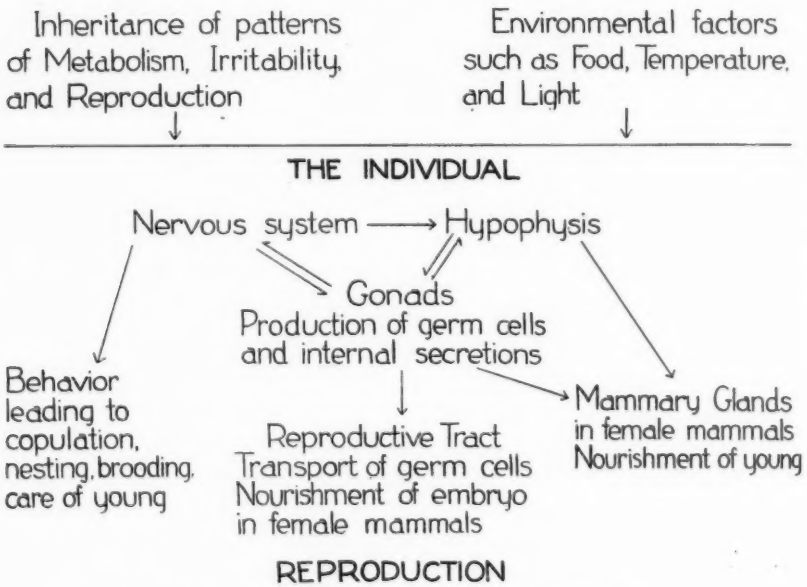

Fig. 1. Factors conditioning the reproductive cycle.

That temperature is an important variable in the environment is shown, for example, by the lengthening of the cycle of laboratory rats when the temperature is lowered (Lee, '26), and the shortening of the cycle of the bat when the temperature is raised. It has been reported that when salamanders are brought to the laboratory early in November and kept at summer temperatures (75°-85° F.) courtship reactions and egg-laying will occur within four days (Branin, '35). The observation that tropical fishes have numerous cycles continuing throughout the year (Turner, '37) while those of the temperate zone have annual or even biennial cycles is relevant in this connection. A few animals (starlings, pheasants, ferrets, and raccoons) have been found to have the reproductive cycle shortened by the lengthening of the daily light period (Bissonnette, '33). Spring breeders will breed in the early winter if given adequate hours of artificial light. Since light is shown to be a factor a pronounced change in the month of breeding would be expected when animals are transferred from one hemisphere to the other. Marshall ('37) reported that sheep bred in England one autumn and shipped to South Africa came into heat again the following May—the late fall of the southern hemisphere. The next year they were in heat in April and the following year in March. A complete adjustment to the new seasons had occurred.

Another variable related to seasonal breeding is the type of food available at different times. The effect of this phase of the environment is almost completely unexplored. It is known, however, that many food-deficiency states condition alterations in the reproductive cycle. Recently, Richter and his students ('38) have utilized the method of self-selection to study the nutritional requirements of rats for growth and reproduction. They find that rats presented with a considerable array of purified foods including proteins, carbohydrates, lipids, salts, and vitamins make consistent selections, maintaining themselves at a high growth level and undergoing regular reproductive cycles. Moreover, during pregnancy and lactation the ratio between foods selected is altered in a specific way as compared with selections for maintenance of continuous nonpregnant cycles. It should be mentioned, also, that vitamin E was identified by its effects in rats on maturation of male germ cells and retention of fetuses in females.

While the effects of diet on differentiation of germ cells and, thus,

on the reproductive cycle may conceivably be direct, it is difficult to consider that seasonal variations of temperature and light are. Students of the alteration of the reproductive cycle by means of light incline to the belief that it initiates nervous impulses which travel to the hypophysis cerebi by way of the hypothalamus (*cf.* Haterius, '37, in this connection.) This gland of internal secretion then reacts in such a way as to hasten the maturation of germ cells. Noble ('37) has conducted experiments on lower vertebrates designed to locate the nerve centers responsible for the pattern of sexual behavior. Striking results have been obtained in suppressing the brooding behavior in several species of tropical fishes by producing lesions in the corpus striatum. Injury to other portions of the brain do not alter this particular pattern. It has been shown, also, that when female fighting fishes have the ovaries removed testes are regenerated (Noble and Kumpf, '37). The pattern of sex behavior is soon changed from the female type to male type. Thus, it seems clear that variations in the reproductive organs can condition behavior patterns by way of the central nervous system. The nervous system plays an important part in the integration of various aspects of the reproductive cycle. Richter ('37) and Hinsey ('37) have discussed other aspects of this interrelation.

Since 1917 the greatest emphasis in the study of the reproductive cycle in mammals has been on the analysis of its hormonal control. Without reference to individual workers, the broad outlines of the results obtained may be summarized in the following way for the female (Fig. 2). Growth and differentiation of egg cells in the ovary are dependent on the secretion of the hypophysis which is carried by the blood stream. As the oöcyte grows it is surrounded by follicle cells which apparently produce a secretion containing an active principle called estrone. This secretion distributed by the blood stream conditions a pronounced increase in the rate of cell division in the reproductive tract. The lining of the vagina becomes thickened and then cornified; this provides a widely used test for the presence of estrogenic substance and, thus, of maturing egg cells. Estrone, also, stimulates cell division in the walls of the uterus and tubes. Further, its presence is correlated in some way with the onset of the period of receptivity to males, or estrus. These effects are conspicuous only in

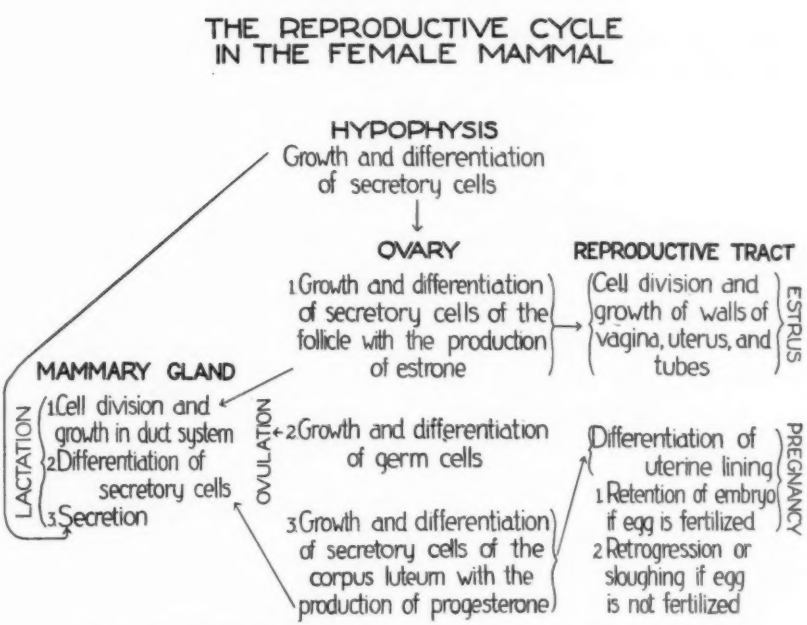

Fig. 2. Hormonal control of the reproductive cycle in female mammals.

the final stages of growth of the follicle and are terminated by its rupture when the ovum is expelled into the Fallopian tube. Then the cells of the ruptured follicle continue their differentiation, give rise to a corpus luteum, and libèrate into the blood a secretion the active principle of which is called progesterone. In the presence of this secretion the endometrium or lining of the uterus differentiates in such a way that nourishment of an embryo can be accomplished by association between the endometrium and the embryonic membranes. If ovulation is followed by fertilization the embryo develops in the uterus thus prepared for its retention. If the egg is not fertilized it disintegrates and the hypertrophied endometrium of the uterus undergoes retrogression or is sloughed. In annual breeders both gonads and tract remain quiescent for the remainder of the cycle. The hypophysis, however, soon becomes active again in those mammals with short estrous cycles and the process is repeated again and again.

The methods of study used in determining the hormonal control of the reproductive cycle are four. (1) The effects of removal of

endocrine glands from test animals are noted. (2) Glands are transplanted from one animal to another. If the graft takes in its host and becomes vascularized, its secretion will be distributed in the blood stream and may bring about noticeable changes. (3) Extracts of organs are injected into experimental animals. (4) Finally, the isolated or synthesized active principle of a hormone can be administered. Operated or sexually immature individuals are usually used for test animals.

The question very properly arises now: Why should a cytologist be concerned with problems of the reproductive cycle. In cells other than germ cells differentiation occurs chiefly in the region outside the nucleus, in the cytoplasm of the cell body. It is the conviction of some cytologists that the differentiation products of a cell, the visible structures which distinguish one type of cell from another, are directly related to the chemical and physical changes that occur in the cytoplasm of these different kinds of cells (Guthrie, '25, and Marza, Marza, and Guthrie, '37). In other words, the activity or physiology of a cell is reflected in part by structures that can be preserved in the cell by certain reagents and studied with a microscope. What occurs during the reproductive cycle is a definite sequence of growth, differentiation, and function of cells in several regions of the body. Analysis of such material can properly be undertaken by cytological techniques.

Bats were selected for a study of the reproductive cycle for several reasons. The bat has an annual cycle; the various phases are in clear sequence with no overlapping such as occurs in animals with short and numerous cycles each year. It can be obtained in large numbers from its natural habitat and when studied at once can be considered to be physiologically normal. The aspects of the typical cycle can be ascertained in numbers of individuals large enough to compensate for the inevitable individual variations. Further, the bats of this vicinity are small; whole organs can be prepared for microscopic examination. During the period since 1930 more than 2000 specimens of six species have been collected and numerous observations made on the natural history (Guthrie, '33 a and b). Some experiments in banding bats in order to determine the course of their migrations have been undertaken but few banded specimens have been recovered.

Certain peculiarities of the reproductive cycle in the bat had attracted the attention of European zoölogists during the last quarter of the last

century (Hartman, '33). It was known that copulation occurred in bats of the temperate zone in the autumn because females taken during the season of hibernation always had living spermatozoa in the uterus. The opinion prevailed that the spermatozoa remained functional throughout the winter and that one of them united with the egg when it was ovulated in the spring. This question of the longevity of the bat spermatozoön has been argued for more than fifty years and has not been settled because the evidence is all indirect. Copulation undeniably occurs in the autumn, but does it occur again? The secluded natural habitat of bats and the poor adaptability to conditions of captivity have made direct observation of copulation very rare, if indeed it has ever been seen. The few reports are so conflicting as to raise grave doubts, but all observations have been in the spring. The female bat does become dormant with spermatozoa in her uterus but does she remain continuously quiescent until the time of ovulation? Little is to be found in the literature with reference to this important point but our observations indicate that bats are not continuously inactive during the winter, and this is true of other hibernating mammals. Why not determine this point by isolation of females in the autumn? They die in our laboratory no matter what attention is given to their care. They die, too, when caged in the caves, which shows again that hibernation is not a continuous dormant period. Miller ('39) has reported for the male Myotis that while the testes are entirely inactive after the middle of September the epididymides are packed with spermatozoa and the accessory glands are fully differentiated from September until April. The male bat is capable of copulation throughout the hibernating period.

The greater part of our studies has been conducted on females of two species of Myotis one of which (M. grisescens) is a summer resident at Rocheport Cave, Boone County, Missouri, and winters as far north as southern Missouri where we obtain it from Marvel Cave in Stone County. The other species, M. lucifugus, is a winter resident at Rocheport Cave. Very few differences, none of them fundamental, have been observed in these two species. The observations to be reported have been made on both living and fixed tissues.

Five or more specific hormones have been postulated to arise in the pars distalis of the hypophysis. The question has arisen as to what cells

present in the gland produce these hypothetical substances, none of which has been obtained in crystalline form. With various single criteria such as an acidophilic or basophilic reaction with a histological stain, or the position of some cell component such as a Golgi net investigators have proposed various classifications of the compactly arranged cells of the hypophysis. Sawyer ('36) correlated the numerous cytological features of growing, differentiating, and functioning cells, such as absolute size, relative size of nucleus and cytosome, and kind, quantity, distribution, and staining reactions of differentiation products. She classified the cells of the pars distalis of the hypophysis

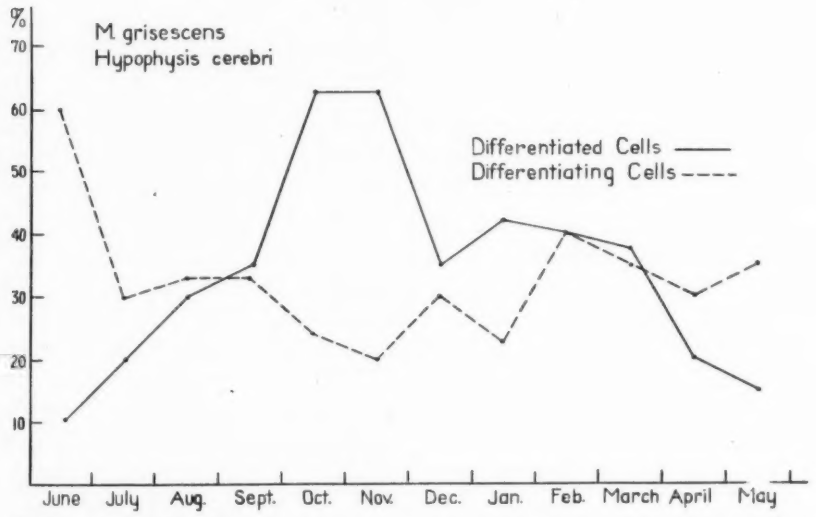

Fig. 3. Seasonal variation in relative numbers of differentiating and differentiated cells in the hypophysis of the bat.

as secretory cells and histiocytes. Secretory cells are found in stages of growth, differentiation, secretion, and degeneration—the usual sequence in the life cycle of a cell. When the numbers of cells which are growing, differentiating, and fully differentiated, i. e., ready to secrete, are estimated for different months of the year marked seasonal variation in function is indicated (Fig. 3). If secretion occurs as soon as a cell is differentiated very few such cells should be seen as compared with differentiating cells. If there are a great many of the latter the rate of secretion should be high. The greater the number of fully

differentiated cells the slower the rate of secretion. The data are taken to mean that secretion is very abundant throughout the summer, reaching its peak in July and August. By September, however, the number of differentiated cells exceeds the differentiating ones and the retention of secretory granules is very pronounced in October and November, the early months of hibernation. Some increase in secretion is indicated in December and from February on an increased rate of outpouring is suggested. This becomes very pronounced in April and May. The secretion of the hypophyseal cells passes into the blood and thence to the cells of various organs where it is conceivable that different parts of the molecule enter into the particular reaction sequences of the several regions. Certainly the cells of the ovary require something from this secretion during their growth and differentiation.

When a female of the genus Myotis hibernates, her ovaries contain only one vesicular follicle. This is the follicle of ovulation and it grows but little until the end of the hibernation period in April. These animals in the autumn exhibit external and internal manifestations of estrus which in other mammals is ordinarily followed immediately by ovulation. Yet ovulation does not occur in the bat until six months later. The simplest explanation might seem to be that a state of complete inactivity exists during this interval and the bat merely awakens in the spring to resume its reproductive activities. Since it seemed desirable to know what was actually happening in the ovary the cytosomal differentiation of the female germ cells themselves as well as of the follicle cells was studied (Guthrie and Jeffers, '38 a). In general the size of the follicle could be taken as an index of the degree of differentiation of the follicle-egg system. Conspicuous waves of variation in numbers of both normal and retrogressing follicles classified in groups on the basis of diameter were found during the hibernation period (Guthrie and Jeffers, '38 b). If the normal follicles are considered in two size groups with respect to the onset of retrogression the characteristics of the seasonal fluctuation are apparent (Fig. 4). On the basis of these findings it is assumed that female bats which are hibernating are in a state of submaximal estrus conditioned by a lowered output of hypophyseal secretion, in some way correlated with the onset of the hibernation period. The variations in number of the non-vesicular follicles are conditioned by differences in threshold of

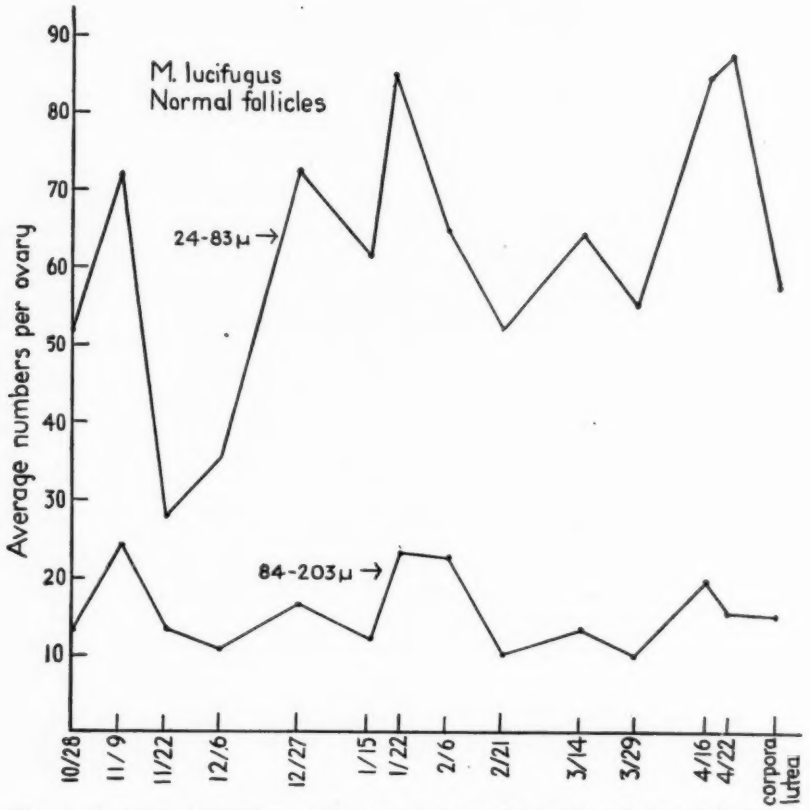

Fig. 4. Variation in numbers of follicles of the bat (M. lucifugus) during hibernation. Retrogression occurs in follicles of the larger size range but not in the smaller.

stimulation to the hypophyseal secretion which is available, but not in constant amounts. The data are interpreted to indicate that the threshold rises as the cells of the follicles become more differentiated so that a very large amount, relatively, of hypophyseal hormone is required to produce a conspicuous response in the vesicular follicle. It is further necessary to note that apparently competition for available hormone occurs with the larger follicles taking all there is if they can get any, unless excessive quantities are present. The possibility that this is simply a question of number of cells in larger as against smaller follicles is likely.

If these hibernating females are in a state of submaximal estrus conditioned by insufficient hypophyseal hormone it should be possible to

bring them to full estrus and induce ovulation by administering hypophyseal extract. That was done in December, January, and February and not only did the vesicular follicle grow and rupture but growth in other size groups (24-43μ and 124-163μ) was conspicuous and follicles with antra appeared (Guthrie and Jeffers, '38 c). These results showing the effect of hypophyseal hormone on smallest growing follicles and on any follicles without antra were at variance with current opinion on this point. Further analysis of the effect of the extract on the follicle-egg system indicated that in small follicles the oöcyte reacted more conspicuously than the follicle cells and that the reverse was true in larger follicles. It had been suggested that the final nuclear differentiation of the egg cell, the meiotic divisions, were conditioned by hypophyseal hormone. When large secondary follicles begin to retrogress the egg cell usually form a meiotic spindle. This suggests that the absence, not the presence, of hypophyseal hormone may be correlated with spindle formation. In injected bats there are fewer oöcytes with spindles; in other words, increase in hypophyseal hormone does not condition spindle formation at the concentration employed.

The follicular population in the ovaries of normal bats beginning with late pregnancy has, also, been studied. Unfortunately there are very few specimens in the earlier stages of pregnancy; the whereabouts of the bats during this period of the year, April to early June, is not known. If the data are presented in terms of the average number of normal growing follicles per ovary a great decrease at the time of parturition followed by a sharp rise will be noted (Fig. 5). There is a decline as lactation occurs and then a spectacular increase as lactation ceases at the end of July (*cf.* Fig. 3). As many as eight follicles with antra may be present early in September. Reeder ('39) who studied the reproductive tract of M. lucifugus found proliferation in the vaginal wall in August and later cornification. By October the uterus and tubes showed the effects of estrone. Both of these conditions are associated with estrus. Females of M. grisescens begin to be inseminated the third week in September. By that time only one tertiary follicle remains and this condition persists until April. The absence of collections in the autumn makes it impossible to compare this graph closely with the one for variations in M. luci-

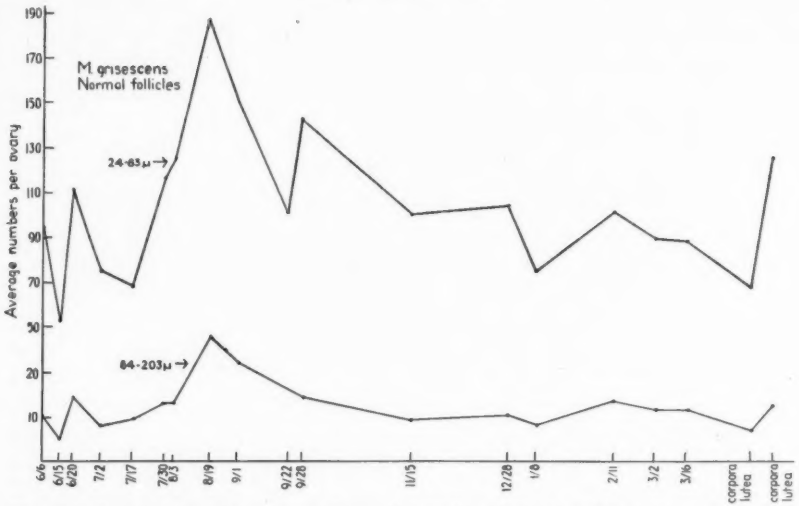

Fig. 5. Seasonal variation in numbers of follicles of the bat (M. grisescens). Retrogression occurs in follicles of the larger size range but not in the smaller.

fugus throughout the winter (Guthrie and Jeffers, '38 b). There are no preovulation specimens. However, a sharp drop is noted in individuals with tubal eggs and a sharp rise within three to four days. M. grisescens ovaries have on the average more follicles than those of M. lucifugus.

These seasonal variations in numbers of follicles are attributed to differences in the amount of hypophyseal secretion available to the ovary. Is there any indication from follicle/oöcyte ratio calculations that the amount of available secretion varies? In figure 6 the ratios for follicles of different diameters are plotted against collections dates. It is clear that the oöcyte and its follicle do not increase uniformly in volume throughout the year. Having seen that an increase in amount of hormone will shift the normal ratio, can these data and those on average follicles per ovary be used to estimate the relative thresholds of response to the hormone? For instance, from July 1 to 17 the follicle data indicate the lowest concentration of hormone for the dates used here, yet the cells of follicles 24-43μ in diameter show a striking gain on their oöcytes; they respond to the lowest concentrations. On August 3 when the follicle counts show that a sharp rise in accessible hormone has occurred, the ratio in most of the size groups is down; the oöcyte responds first. On August 19, when counts indicate the greatest

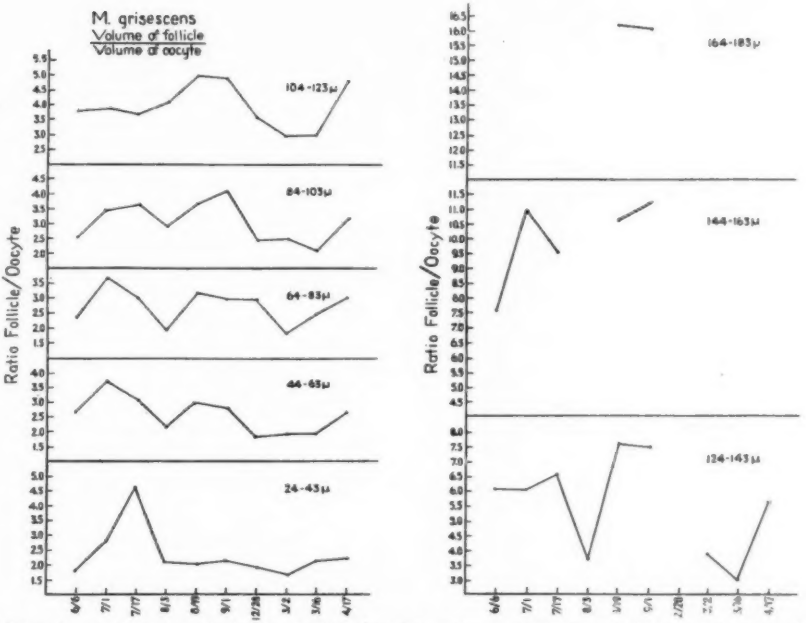

Fig. 6. Seasonal variation in follicle/oöcyte ratios in follicles of different sizes in the bat (M. grisescens).

availability of hormone, the ratio in follicles 124-143μ in diameter has increased from 1:3.7, on August 3, to 1:7.6. These data point the way to a fuller understanding of growth and differentiation in the egg-follicle system.

It is apparent that the bat is a valuable animal for study of the factors conditioning the reproductive cycle. Since it has this long period of submaximal estrus, unoperated adults can be used for hormonal experiments. In addition an animal without a temperature regulating mechanism and with conspicuous shifts in manner of utilization of its food becomes a test object for a variety of other stimuli which may condition the reproductive cycle. For example, we have found that after the middle of February ovulation will occur when bats are brought to the warm laboratory from the caves. During the second half of March ovulation will occur within 24 hours under such conditions. Thus, it may be possible in the bat to separate factors in the complex conditioning the reproductive cycle that might not be suspected in a more closely integrated organism.

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APPLICATIONS OF REPRODUCTION PHYSIOLOGY TO LIVESTOCK BREEDING*

By

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The efficient operation of a livestock breeding enterprise requires not only an understanding of the principles of nutrition, sanitation, economics, and farm management, but a knowledge of the physiology of reproduction.

Females in the breeder's herds and flocks mate only at certain times. When females will accept the male they are said to be in estrus (or in heat). Do mares cows, ewes and sows express such a desire *every* month in the year? Is there an optimum time for impregnation or breeding? If so, when is that time with respect to estrus? How long is estrus? How long from one estrus till the next? In addition to the behavior reaction of estrus what internal changes characterize this period? What irregularities are there? Can one effectively and profitably induce estrus? Pregnant females frequently require different care in the efficiently operated enterprise, hence the importance of an early pregnancy diagnosis. But how is this done?

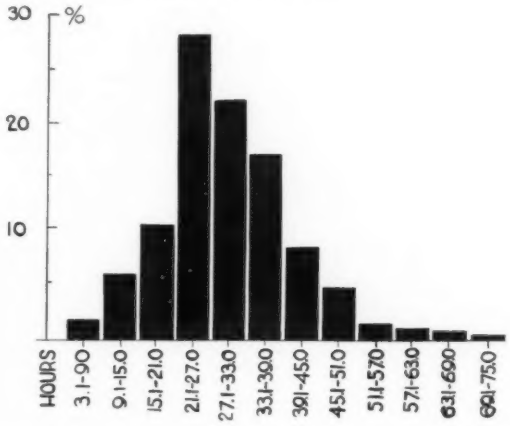

Fig. 1. Frequency graph showing the duration of estrus for 344 ewes, based on 1,235 observed periods of estrus.

*Contribution from the Department of Animal Husbandry, Missouri Agricultural Experiment Station, Journal Series No. *619*.

Is a male either entirely fertile or sterile or do various levels of fertility exist? How can one estimate the fertility level in the male? Are there seasonal fluctuations in male fertility? What constitutes excessive sexual use of the male? What techniques can be used to avoid excessive use and yet impregnate a number of females?

Answers to such queries as these provide valuable information to the livestock operator, yet in spite of the age-long need for such it is only recently that investigators have revealed much specific data.

THE FEMALE

The duration of estrus in the ewe ranged from 3 to 73 hours with a mean of 29.33 hours as shown in a study at the Missouri Agricultural Experiment Station (see figure 1). A range of about 9 hours on each side of the mean (21-39 hours) included 73% of the periods; 92% fell between 15 and 45 hours. There was a tendency for a larger number of estrual periods to be initiated between midnight and noon than between noon and midnight. The length of the estrual cycle in the ewe

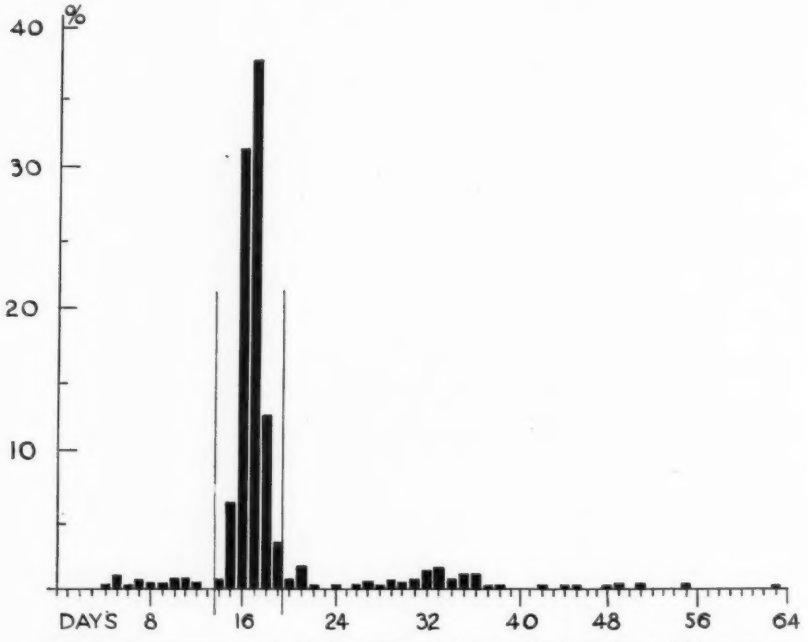

Fig. 2. Frequency graph of the length of 1,038 estrual cycles on 299 ewes.

(from the onset of the one estrus to the onset of the next) varied from 4 to 63 days; 90% of the 1,038 cycles observed ranged from 14 to 19 days (see figure 2).

During the course of the breeding season there was an increase in the length of the estrual cycle. The duration of estrus was shorter during the first and last months of the breeding season. Sterile copulation tended to shorten the duration of estrus but had no effect on the length of the estrual cycle. Ewes on a low plane of nutrition had a shorter breeding season and longer estrual cycles.

The duration of estrus and of the period from the end of one estrus till the beginning of the next is tabulated for each class of stock in table 1.

Sheep are seasonal breeders. So are horses but to a less extent, and with cattle and swine we hardly recognize breeding seasons from the biological standpoint with the improved stock of today. In the Corn

TABLE I

DURATION AND FREQUENCY OF ESTRUS, TIME OF OVULATION AND GESTATION IN FARM ANIMALS IN ORDINARY CONDITION

	In Estrus for	If not Impregnated Estrus will occur in	Time of Ovulation	Optimum Time To Mate In Estrus	Gestation (in days)
Mare	1, **3–7**, 27 days	**13–18**–24 days	2 days before end of estrus till 1 day after estrus	3–5–7th day	310–**334**–350
Cow	12–**16**–18 hours	18–**19**–22 days	24–40 hours after estrus begins	Well in estrus—and 6 hours later	278–288
Ewe	**20–30–42** hours	13–**15**–16 days	1 hour before estrus ends	After 12 hours	144–**147** (Fine Wools — 152)
Goat	20–**39**–80 hours	11–**18**–24 days			143–**148**–157
Sow	2 days (gilts) 3 days (sows)	17–20 days	Early 2nd day of estrus	2nd day	111–**112**–115

NOTES

1. Heavy figures indicate the most common duration.
2. The foal estrus in mares usually lasts five days, but may range from 1-10 days.
3. Mares usually come into estrus 5-10 days after foaling.
4. Hampshire ewes stay in estrus about 31 hours; Shropshires, 26 hours; Southdown, 24 hours; in lambs of all breeds usually less than one day.
5. Best time to hand breed is soon after the middle of estrus.
6. Ewes produce the largest number of lambs when they conceive at the very beginning of the breeding season.
7. Most sheep are seasonal breeders. In Missouri the breeding season is from August to January. Rambouillets and Hampshires first, the Southdowns and Shropshires.
8. Sows come into estrus 3-4 days after weaning a fair-sized litter; earlier if litter is small.
9. To estimate the length of the estrual cycle for any species listed add the figures in columns one and two.

Belt section of the United States the Hampshires, Southdowns, and Shropshires come into the breeding season the latter part of August and September and, failing pregnancy, estrus will recur until December and January, for the most part. As to the factors that are significant in initiating the onset of the breeding season, there is some conjecture. There has long been prevalent among sheep men the thought that the ewes will "come in" the first cool nights of the fall. No doubt this is based on a coincidence in the more northern sections. However, many in Missouri cannot recall many really cool nights in August and September. In order to investigate the effect that temperature might have on the onset of the breeding season, we have subjected ewes to 12 or 24 hours each day in rooms cooled by ice to 44-48°F. (70°F. minimum and 95°F. maximum outside temperatures). Some of the "cooled" ewes were given freshly cut Sudan grass and some of the ewes not cooled were given some of the fresh grass. The onset of the breeding season was the same in all groups (less than a day's variation between the groups). This work was repeated another season with the the same results. There is a difference amongst breeds with respect to the onset of the breeding season; our Hampshires come in first, then the Southdowns, and finally the Shropshires. Rambouillets, the western breed of fine-wool sheep, have their season in July in some of the hotter sections, as for example Texas. In this connection the observation reported by Marshall of Cambridge is interesting. A group of 21 Southdown ewes from Great Britian arrived in Cape Province, South Africa, January 1933. All but one lambed within a month following January 23, 1933. The lambs were weaned and the ewes bred beginning May 21, 1933. The following year they were bred late in April. In 1935 and 1936 they were bred in March. Thus the South African fall became the breeding season for these ewes, as the northern fall had when they were in Scotland. (F. H. A. Marshall, Sexual Periodicity and the Causes Which Determine It, Philosophical Transactions, Royal Society, London, B 226, P. 449, 1936).

The process of ovulation, (the shedding of the egg) has been studied in this laboratory at 219 laparotomies on sheep. Follicles were actually observed at the time of rupture in 36 cases. The pre-ovulatory period was determined within rather narrow limits in 79 estrual periods.

Mature follicles, straw-pink in color, usually appeared darker and more vascular than the other follicles, but could rarely be distinguished more than 4 hours before they actually ruptured. The extent to which the follicles swelled out was variable. Some protruded as much as 8 mm from the surface. As the follicles swelled the membranes over the surface became thinner and more transparent. About an hour before rupture a small round area, usually near the center or peak of the follicle, became very clear or transparent. A few minutes before rupture one or more tiny cones or projections from 1 to 3 mm in height swelled out from this clear area. The break occurred at the apex of the cone. Following rupture, there was a *gradual* collapse of the follicle. The extruded material was thin at first but became viscous within 2-3 minutes after ovulation and could be drawn out in strands 8-12 mm long. A gelatinous plug, usually blood streaked, formed at the rupture point. Histological studies of the sheep ovary have clarified greatly our ideas of this remarkable phenomenon of ovulation (see Virgene Warbritton Kavanagh and Fred F. McKenzie, Histological Observations on the Mechanism of Ovulation, Anat. Rec., V. 73, 3, Suppl. 2, p. 30, 1939).

Time of ovulation in the ewe varied from earlier than 12 hours after the onset of estrus to later than 41 hours after the onset. In the main it occurred about 1 hour before the end of estrus. Double or twin ovulations varied from 0.37 to 7.3 hours (a mean of 1.73) between each of them. The pre-ovulatory period (from onset of estrus to ovulation) was longer when two follicles ruptured than when only one did, but the length of estrus and the estrual cycle were unaffected. There was no definite pattern for the order of ovulation between the two ovaries. The pre-ovulatory period decreased during the breeding season, and the ovulation rate was lower during the latter part of the breeding season. Ovulation without estrus occurred in 16 or 17 cases approximately 1 cycle prior to the first estrus of the breeding season, and in 9 of 15 cases approximately 1 cycle after the last estrus of the breeding season. Sterile copulation had no effect on the pre-ovulatory period or the rate of ovulation. Ewes on a low plane of nutrition had shorter pre-ovulatory periods and lower ovulation rates.

The sow has been studied, as has the sheep. Estrus in gilts (immature sows) lasted 45 hours, sows 65 hours, the cycle 21-22 days;

ovulation time 32-37 hours. All ova were discharged within 9-15 minutes after the first.

A somewhat comparable study has been made more recently on the mare by Frederick N. Andrews and this author working for the most part at the United States Range Livestock Experiment Station, Miles City, Montana. Estrus ranged in length from 1 to 37 days with a mean of 5.28 days, 74.5% of the periods lasting 2-8 days. Three days was the most common duration of estrus for light mares, whereas 5 days was most common for draft mares. The length of the estrual cycle for mares of both types was 20.7 days (range 10-37 days), 75.6% falling between 17 and 24 days.

Most mares exhibited their maximum sex desire within two days of ovulation, estrus terminating near the time of ovulation (as determined by rectal palpation) (see figure 3).

What has been termed "split estrus" was characterized by an initial estrus of one or more days followed by a non-receptive period of usually 1-2 days, and a subsequent return of estrus for one or more days. Ovarian follicles continued development during the non-receptive period and ovulated after the mares returned to the estrus condition. Thirty-one per cent of the mares of the herd exhibited "split estrus". Seventy-eight per cent of the mares bred during "split estrus" conceived. "Physiological estrus" or silent heat was manifested by the ovarian, uterine and vaginal changes characteristic of estrus, but they were not receptive to the stallion. This was observed more frequently during March and April than later. Ten per cent of the mares showed this condition and 62.5% of those bred became in foal. Ovulation was found to occur in some cases 2-5 days after estrus. When artificially inseminated 75% of these mares conceived. The artificial rupture of follicles of a persistent or normal type was followed by normal estrus, and mares bred then conceived. Follicular fluid recovered ranged from 6 to 112 cc. in volume and from 20 to 75 gamma of estrone per cc. Most follicles were 2-3 cm. in diameter on the first day of estrus and 3.5-5.0 cm. on the day preceding ovulation. Ovulation occurred in the left ovary in 60% of the cases observed. No definite pattern for the order of ovulation between the two ovaries was observed. Although more than one follicle was often palpated on one or both ovaries during estrus, double ovulation occurred in only 3.8% of the cases observed.

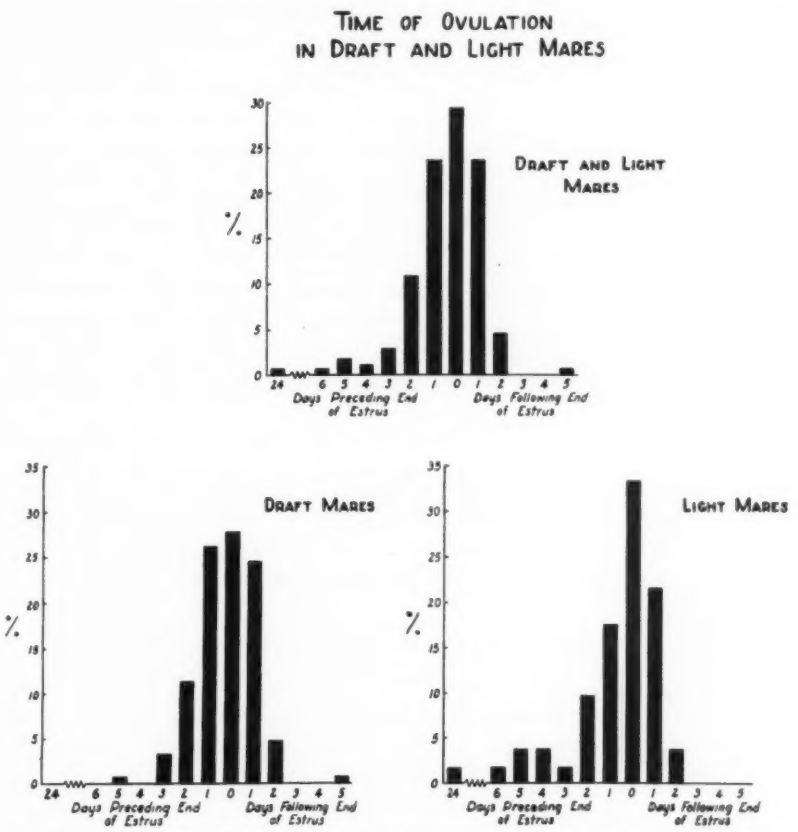

Fig. 3. Frequency graph of the time of ovulation in 45 draft and 35 light mares.

The greatest vascularity of the vagina and cervix was near the time of ovulation and lowest 5 to 10 days after estrus. As early as the third week of pregnancy the vaginal mucosa had a blanched appearance that was of considerable value in the physical diagnosis of pregnancy. The secretions recovered from the vagina and cervix were most abundant and thinnest (watery) near the time of ovulation. The secretions were alkaline throughout the estrual cycle and were more alkaline (potentiometric determinations) during estrus than during interestrus. The cervix was most relaxed just before ovulation (see figure 4). Both rectal and vaginal temperatures were slightly higher (0.1-0.25°F.) during estrus than during interestrus, and those of lactating mares

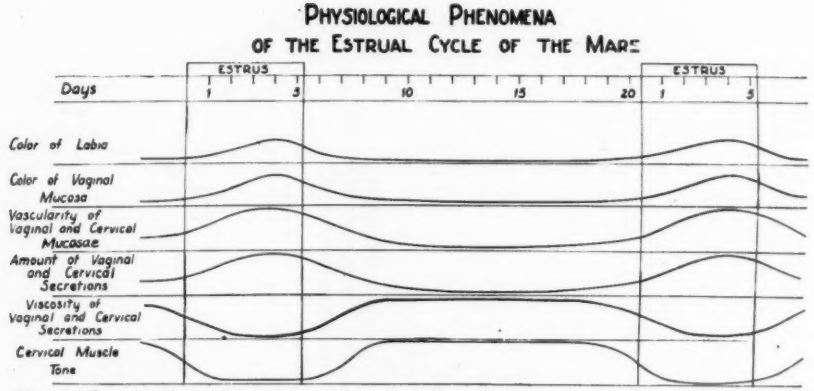

Fig. 4. Graphical representation of the physiological phenomena of the vagina and cervix during the estrual cycle in the mare.

higher than those of dry mares (.06-.21°F.). Seventy-two per cent of all mares conceived. Of the lactating mares 73.1% conceived, and of the dry mares 70.7%. Fifty per cent of those bred during foal estrus conceived. Forty-seven per cent of the maiden mares bred conceived. conceived.

Under many farm management practices livestock are so handled that they are not free to mate at will, but rather males and females are brought together at times convenient to the operator. This is called hand breeding and for best results it is well to know when is the optimum time to mate in order to insure conception.

The time of the shedding of the egg or ovum (a process referred to as ovulation) has been investigated in most species of farm animals. It is generally conceded by livestock men and scientists that insemination (or the depositing of the male germ cells or spermatozoa in the female tract) should precede by a brief interval the time of ovulation. This allows for the transport of the sperm through the female tract up to the region where fertilization will take place, the sperm to have arrived in appreciable numbers by the time the egg has beeen liberated and migrated to this same area (upper portion of uterine tube). The optimum mating time for each class of stock has been set forth in table 1.

The period of gestation or the duration of pregnancy has been determined in a large number of individuals and these figures appear in table 1. Many important physiological changes occur in the individual once pregnancy begins. Two phenomena have significance to the

livestock man and are mentioned here. In the mare, following about the fortieth day of pregnancy, there is an increase of a principle in the blood, that when injected into test animals, stimulates the ovaries (a gonad-stimulating hormone). This principle reaches a remarkably high level and maintains this level till about the 100-120th day of pregnancy when it rapidly diminishes. In the urine of the pregnant mare there appears about the 86th day a principle that is estrus-producing in effect. This hormone persists throughout the later stages of pregnancy. (See figure 5). Thus, one may assay the serum of the mare bred 45 to 110 days and test for pregnancy, or assay for the estrus-producing principle in the urine of the mare bred 100 days or more and determine if she is pregnant by this means.

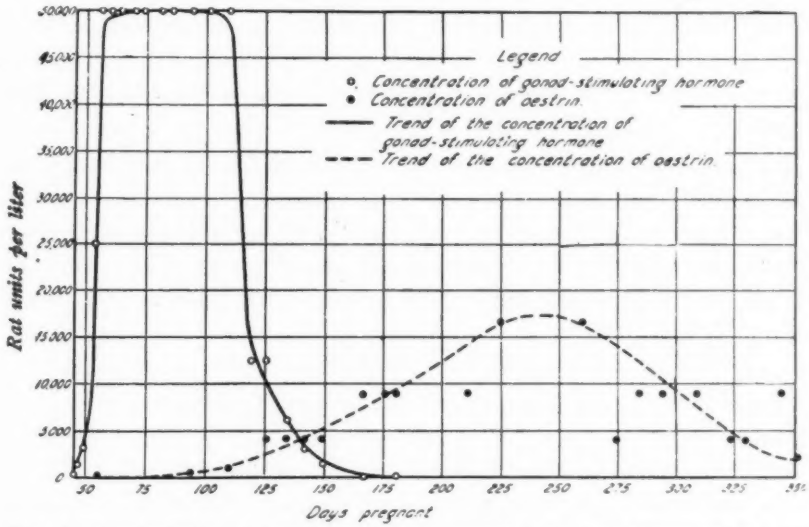

Fig. 5. Concentration of gonad-stimulating hormones in the blood serum and of estrin in the urine of a mare during pregnancy (from H. H. Cole and F. A. Saunders, Endocrinology, Vol. 19, No. 2, March-April, 1935, pp. 199-208).

As shown above in the case of mares certain females fail to show outward signs of estrus. The ovaries of some of these will respond to certain stimulating principles. A sheep pituitary preparation (APL of Jensen-Salsbery Laboratories, Inc.) has proven efficacious in mares even when the ovary seemed not to be going through its cyclic changes. Pituitary implants have stimulated a sow with hypotypical (under-de-

veloped) ovaries to come into estrus. At the next succeeding estrus the sow conceived. Pregnant mare serum (gonadin, Cutter Laboratories) has potency in inducing estrus in ewes out of the breeding season, but has limited value during the months when sheep normally breed. This product and pregnancy urine (of women) (Antuitrin-S, Parke, Davis, and Company) have definite value in inducing ovulation in mares if administered at certain stages of follicular development. Uusally this is after estrus has begun. The practical application of this procedure is to shorten the period of estrus in the mare and hence reduce the number of inseminations required. The above statements are based on observations made in this laboratory. Other investigators have obtained somewhat similar results, notably Mirskaya, et al (Mirskaya, L. M. & V. V. Patropavtovsky, Reduction of the normal duration of heat in the mare by aid of prolan. (Russian) Probl. of An. Husb., No. 3, 1938) and Cole and Miller (Cole, H. H. and R. F. Miller, Artificial induction of ovulation and oestrum in the ewe during anoestrum. Am. Jour. Physiol. V. 104, 165-171, 1933).

Endocrine therapy offers much for the livestock operator, but it is a new and difficult field, and rapid developments are to be expected. It is one matter to induce estrus; it is another to induce the ovulation of a fertilizable egg and synchronize development in the tract that will afford a favorable environment for sperm.

THE MALE

Males differ in their ability to impregnate females. For example, one bull might average less than two copulations per conception, and would be considered a highly fertile animal, whereas another bull bred to the same cows another season might require 2-3 services per conception. Frequently, bulls require more than three, and would rate therefore in the low fertility class. The same holds true for horses, sheep and swine. In estimating the reproductive capacity of a male it is important to consider: The breed or strain, the season of the year, the number of matings just prior to sampling the semen, sperm concentration, total number of normal sperm, and if facilities permit, the oxygen consumption rate of the sperm.

In certain wild forms, such as the deer, there has long been recognized an active breeding season or rut. Stallions, bulls, rams and

boars commonly mate throughout the year when opportunity is afforded, and young of these species have been born at all seasons. However, farming practice has largely limited the horse and beef cattle breeding seasons to the spring, sheep and swine to spring and fall, and only certain breeds of sheep will breed in the spring.

To what extent the reproductive capacity of the male fluctuates with the season has yet to be investigated for most species. Observations made in this laboratory have had to do chiefly with sheep and swine. In figure 6 one can note the seasonal fluctuation of the Shropshire rams in sperm numbers from a high in the fall to an extreme low in summer. Not only did the number of sperm drop in July and August under Missouri conditions, but the concentration of sperm was very low. The volume of semen that may be ejaculated in the same time interval was lowest in late winter and early spring and rose to the year's high in early fall. The proportion of morphologically abnormal sperm to normal sperm was greatest in August.

Two things stand out in this group of graphs (figure 6). First, the marked drop in potential reproductive capacity following the onset of hot weather, and second the genetic or inherent differences in this respect between the two breeds observed.

By allowing access to a cool room where the maximum temperature during the summer months was 80-82° Fahrenheit rams have shown less fluctuation. Thus in the Shropshires so cooled total sperm numbers and total number of normal sperm maintained a steady and high level instead of the marked drop shown by the controls. Hampshire and Rambouillet rams subjected to the same treatment showed the same trend but to a less extent. That is, some Hampshire rams showed a greater capacity to withstand the high summer temperatures than did other Hampshire rams, and the Rambouillets withstood such damaging influence more consistently than rams of any of the other breeds studied. In other words, summer temperatures of 82° Fahrenheit and above adversely affected the reproductive capacity of some sheep. We must therefore recognize genetic differences in ability to withstand high environmental temperatures.

Shearing the wool from the rams in early July and again in early August was of material help in conditioning the rams for an early breeding season. And when access to cool quarters was allowed in

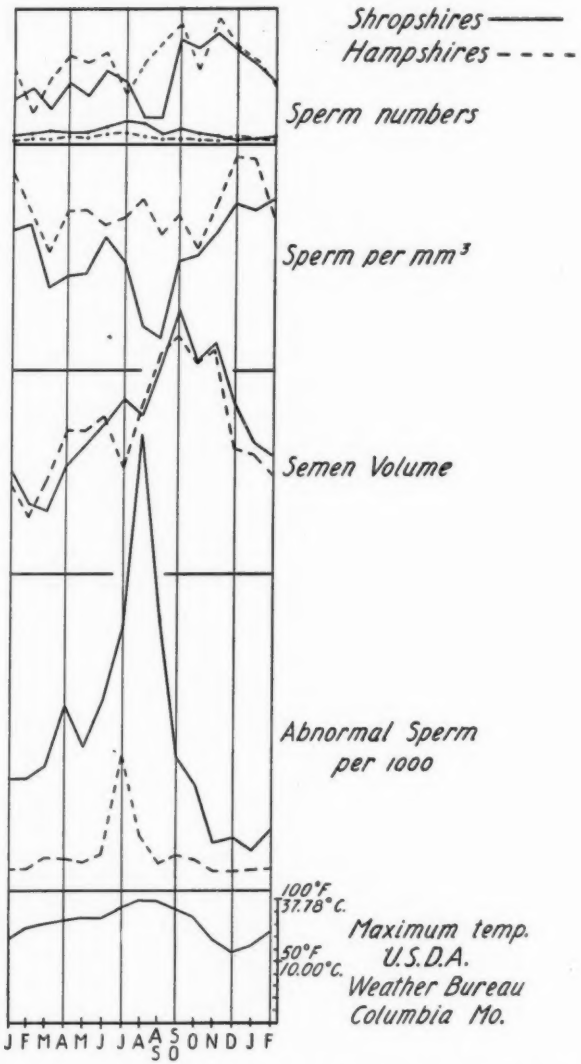

Fig. 6. Seasonal fluctuations for 14 months showing for two breeds of sheep the total and abnormal sperm numbers, concentration of sperm, semen volume, proportion of abnormal sperm, and maximum temperatures for the same period.

addition to the summer shearing the reproductive capacity of the rams so treated was further enhanced. This showed up particularly when the rams were subjected to tests designed to temporarily exhaust them sexually.

Rams cooled and sheared respired at a much lower rate than did the controls (75 per minute as compared with 125).

Whether the effect of high temperatures on reproductive capacity is directly upon the testicles is not known. When the scrotum of a highly fertile ram was blanketed so that the scrotal temperature was raised 4° Fahrenheit above the normal of 95° Fahrenheit (normal rectal temperature 104° F.) an aspermatic condition was developed within three weeks, and semen smears indicated a disturbance four days after the insulation was applied. Figure 7 shows photographs of the seminiferous tubules of testicles so treated four days and one week. Thus, is seen what happens when the scrotum is prevented from maintaining the testicles at their normally sub-rectal temperature even in normally cool or cold seasons of the year.

The relation of frequency of copulation to fertility in the male has economic significance. There is much individual variation here. Once daily for the mature horse, 1-3 daily for the bull, and 4-6 for the ram would seem to be safe recommendations, although reliable experimental data on this subject are yet to be accumulated. In the yearling boar (12-16 months) there has been noted a reduction in the length of time sperm retained motility as the boar was permitted to copulate at 72-hour, 48-hour, 24-hour and 12-hour intervals (see figure 8). When ejaculations were made at intervals of 48 hours or longer, the semen volume remained above 200 cc, the number of sperm per cu. mm. above 100,000 and the total number of sperm per ejaculate exceeded 20 billion. Furthermore, the number of abnormal sperm did not exceed 100 per 1000. Repeated ejaculations at intervals of 24 hours or less reduced semen volume below 200 cc., sperm concentration to 15,000-50,000 per cu. mm., and total number of sperm to 2-5 billion. The number of sperm abnormalities increased to more than 200 per 1000 sperm. At 12-hour intervals some of the boars temporarily lost their desire to mate. Sperm with a cytoplasmic cap appeared in considerable numbers after extreme sexual activity. Observations on the effects of frequent ejaculations on semen volume, sperm numbers, duration of sperm motility and sperm

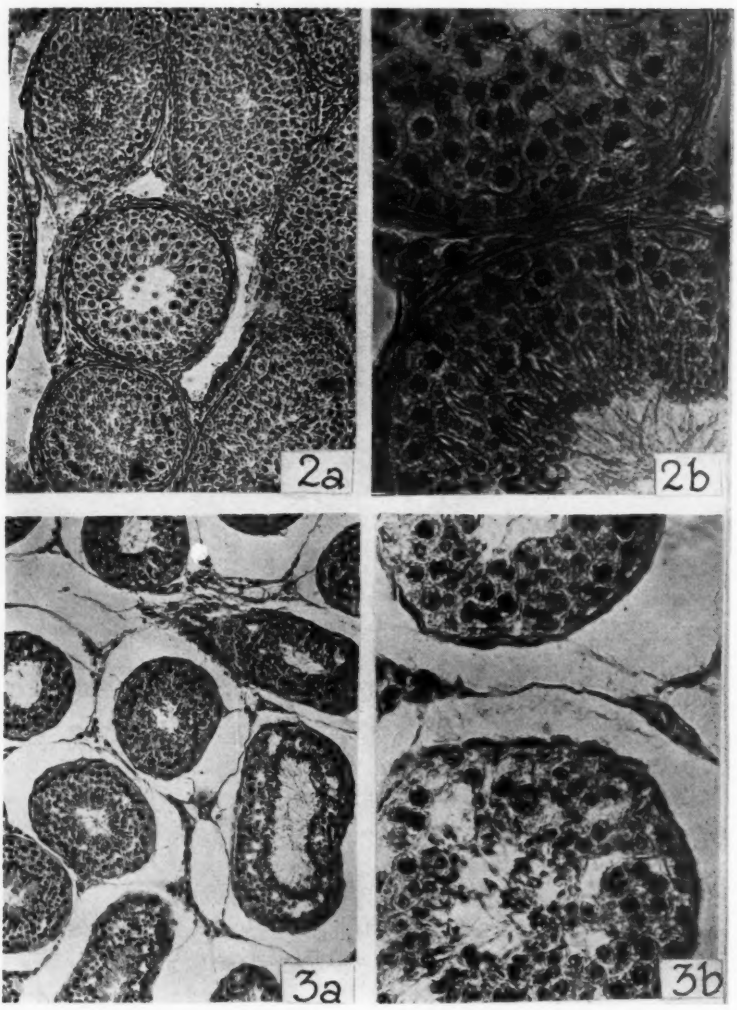

Fig. 7. 2a, 120X and 2b, 360X. Section of a ram testicle after four days of scrotal insulation which raised the temperature of the scrotum from 95°F. to 99°F. (rectal temperature 104°F.). Note the absence of sperm from some tubules, sloughing of cells, clumping and disintegration of cells, and cytolysis of some cells in the germinal epithelium.

3a, 120X and 3b, 360 X. Section of another ram testicle after one week of scrotal insulation. Note the marked shrinkage of tubules, edematous condition, sloughing and clumping of cells, cytolysis, pycnosis, and the chromatoid condition of many nuclei.

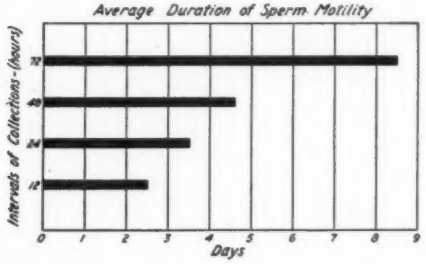

Fig. 8. The relationship of frequency of ejaculation to the duration of sperm motility in the boar.

morphology indicate that yearling boars should not be used more often than once in 24 hours, and that best results might be expected at 48-hour intervals if the breeding season is to extend over a period of two weeks or more.

For a long time the collection of semen from horses and some other forms has been practiced and this material placed in the female tract thus inseminating her artificially. Recently considerable interest has developed, notably in the U. S. S. R., in inseminating a large number of females with the semen of one ejaculate thus increasing the use and efficiency of valuable sires. Different methods of collecting the semen have been developed, chief of which is the artificial vagina. This consists of a thin rubber tube around which is placed a casing to hold the thin inner tube in position and to afford the right amount of pressure. In some devices warm water is put between the inner tubing and the casing as males of most of the species demand that the instrument be a little above body temperature at the time of use. Thus it is possible to collect

TABLE II
CHARACTERISTICS OF SEMEN

Animal	Volume per Ejaculate (cc.)	Sperm Concentration (per cu. mm.)	pH	Volume of Undiluted Semen Recommended per Artificial Insemination (cervical) (cc.)
Stallion	40–320 (**75–150**)	30,000–800,000 (**60,000**)	7.0–7.8	10–30
Bull	2.0–14.0 (**3.0–4.0**)	300,000–**800,000**–2,000,000	6.5–7.5	0.5–1.5
Ram	0.5–2.0 (**0.8**)	500.000–**1,000,000**–6,000,000	6.2–6.8	0.1–0.2
Boar	125–500 (**200**)	25,000–**100,000**–1,000,000	6.8–7.2	100
Dog	2–19 (**7.0**)	1,000,000–9,000,000	.....	.....
Rabbit	0.4–6.5	100,000–2,000,000	6.8–7.5	.25–1.0
Cock	0.1–1.5 (**.3.–8**)	80,000–6,000,000	6.8–8.3	.05–0.1

Heavy figures indicate most common levels, usually satisfactory.

and study semen without having it contaminated, especially avoiding mixture of female secretions of the vagina and cervix with the semen. The characteristics of the semen of farm animals are set forth in table 2. Certain processing techniques have been developed by which semen can be stored, shipped if necessary, and used at a distant place, or divided into several portions and used to inseminate a much larger group of females than would have been possible naturally.

Under natural conditions semen is ejaculated, deposited in the lower female tract, and sperm mixed with the fluids of the male accessory glands and also with the secretions of the female tract are greatly activated and make their way up the tract, one or more sperm to fertilize the egg(s) that may be shed, the others to perish. Preservation of sperm outside the animal body involves conserving their energy by cooling, by lowering the pH, by maintaining as nearly aseptic conditions as possible, avoiding sunlight and mixture with air, water, and many chemicals.

Properly done, artificial insemination can minimize the spread of infection in herds and flocks and can make for increased efficiency in livestock production, under certain conditions; improperly done it can accelerate the rate of loss through disease and depletion of sexual health. For a complete discussion of the subject the reader is referred to "Artificial Insemination in Livestock Breeding", soon to be published by the United States Department of Agriculture.

SUMMARY

A body of data is now available giving us the length of estrus, of the estrual cycle, time of ovulation, the season of breeding in all our farm animals. Certain irregular phenomena, such as "split estrus", "physiological estrus", and ovulation after estrus, are recognized and can be handled rationally. The optimum time to mate individuals can now be estimated.

The length of gestation is known for each species of farm stock. Pregnancy in the mare has always been difficult to diagnose, but at least two biological assay techniques are available whereby the blood and urine of mares may form the basis for pregnancy testing.

Progress is being made in inducing ovulation, and when reliable methods are developed it will be a relatively simple matter to synchro-

nize ovulation and insemination and thereby minimize waste (time, effort).

We recognize degrees or levels of fertility in the male and techniques are available for estimating these levels in advance of the actual breeding season. The adverse effect that high summer temperatures may have on male fertility has been recognized and in those individuals susceptible to such heat it has proved worthwhile to alleviate or entirely avoid the situation by summer shearing of rams, and if possible, allowing them access to cool quarters and cool drinking water. Inherent differences in their ability to withstand excessive heat have been noted in rams and the enterprising livestock breeder might find it worth his while to select deliberately those individuals that adapt themselves to hot environment and propagate their kind.

To limit the number of copulations allowed a male makes for improved semen quality, conservation of the herd sire, and a higher percentage of conceptions. If further conservation of his energies is necessary, and it is desired to extend his use to numbers of females beyond any possibility of natural insemination, one may resort to artificial insemination and perhaps storage and dilution of the semen.

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PHYSIOLOGICAL FACTORS IN REPRODUCTION OF PLANTS

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Plants, like all other living things, reproduce for the simple reason that they die. Reproduction, of course, does not prevent them from dying, in fact, it often speeds up the time of its arrival, but it does prevent organisms from dying out. The group, not the individual, thereby is preserved.

Fundamentally, reproduction is the same in both plants and animals. In *modus operandi* there is, however, a great difference between the two, if we limit the comparison to higher forms of both groups. Being less differentiated organically and functionally, plants reproduce more readily asexually, that is, without gamete formation and union. But, excepting the lower phyla, with all of them sexual reproduction is the most important process of securing a new generation. Asexual reproduction, with which everyone is familiar, will not be discussed here. It is a mode of regeneration of an individual from a part of itself. Essentially, it is the same as experimental regeneration from parts of the body of lower animals, the flatworm *Planaria,* for example. Except for chimeras and mutations, asexual reproduction in plants is a means of perpetuating the germ plasm, thus maintaining the same genetic constitution in successive generations.

One of the main, if not the chief purpose of sexual reproduction is the continuous introduction of great variability within the group and between groups of plants, thereby leading to their increased adaptability to various and changing environments. Further comment on this feature would lead us directly into genetics, which of course is not the subject of this discussion.

The physiology of sexual reproduction is still a very much neglected field of study of the functional life of plants. Very few of our textbooks of plant physiology consider it at all, and what attention is sometimes given to the matter is mainly by way of description of its morphological features, with little emphasis on the metabolic aspects. Yet the development of all higher plants and the perpetuation of the species is intimately linked with the formation of flowers, with fertilization, and the growth of seeds and fruits.

The main subdivisions of the subjects

Any process as complex and inclusive as sexual reproduction in higher plants can be best discussed when it is subdivided into its major constituent phases, of which the following may be arbitrarily recognized:

a) Induction of the reproductive state, sometimes referred to as "ripeness" of the sporophyte to "flower." The initiation of floral primordia.

b) Development of the sexual and accessory reproductive organs—parts of the flower.

c) Meiosis, growth of ♂ and ♀ gametophytes, and the formation of micro—and macrogametes.

d) Fertilization or gametic union and the production of endosperm.

e) Growth of the zygote (embryo), seed and fruit development, and its effect on the mother sporophyte.

For various reasons which need not be mentioned here, but largely because of limitations in satisfactory information, a more detailed discussion will be made of some of these phases than of others. And, in order to make "the story" more complete, consideration will be given not merely to the writer's own investigations but also those of others, though manifestly no complete survey of the field is possible here because of lack of space.

Theories and experimental evidence on initiation of the reproductive state:

Since the genetic constitution of a plant determines not only the type of reproduction but also to a considerable extent the time of its onset, it was thought for a long time that sexual phenomena in their entirety were under control of the germ plasm. Environmental factors, though being able to modify the action of genes, were not believed to be able to change, or delay or inhibit greatly the "inherent" ontogenic developmental sequence of a particular type or kind of plant. The general enthusiasm for the striking, almost mechanical features of Mendelian inheritance and the ready acceptance of Weismann's germ plasm theory, precluded for some time any consideration of what evidence there was of physiological influence upon and control of development. The seat of the genes was supposed to be insulated from the environment to an extent where the idea was almost "sanctified". "Physiolo-

gical genetics" was not yet under consideration, studies of the metabolism of development of plants were in their infancy, and interest in physiology of reproduction was yet to come.

With the rapid growth during the current century of plant physiology as a popular discipline of the botanical science, all phases of the life of plants have come under consideration, including lately also the physiology of flower, seed and fruit production.

There are two major concepts extant at present as to the cause of initiation and maintenance of sexual reproduction in plants: (a) The nutritional theory, promulgated by Klebs, and (b) the "special substance" (hormone) theory advanced by Sachs.

Nutritional theory of reproduction:

By growing plants (*Sempervivum, Sedum, etc*Q under various lights and temperatures, Klebs (20, 21) was able, more or less at will, to maintain continuous vegetative growth and to induce reproduction prematurely. He came to the conclusion that a balance between carbohydrates and soil nutrients (later reduced to soluble carbohydrates and soluble nitrogen) is the primary internal cause of initiation of the flowering state. Klebs made three very limited analyses of expressed sap of leaves. The chemical results showed that plants which subsequently (after three months) flowered abundantly had the highest concentration of sugars and lowest concentration of nitrogen and soluble ash. Those that did not flower were just the opposite in chemical composition, while others, flowering but little, were intermediate in this respect. Since the data for sugars and ash were inconsistent, he concluded that it is not the actual quantities that are significant but a balance (ratio) between them.

Kleb's concept remained more or less dormant till Kraus and Kraybill (24) reemphasized the idea and gave it a renewed impetus. They tried to establish the carbohydrate-nitrogen composition in causal relationship with vegetative development and sexual reproduction of plants, using the tomato as an illustration. In this case (tomato) reproduction was considered in its totality, though the authors suggest that "the conditions for the initiation of flower primordia and even blooming are probably different from those accompanying fruit setting."

Almost all of the supporting and some of the contradictory evidence

of the C/N relationship to reproduction unfortunately has come from analyses of plants in advanced stages of reproduction. It has never been shown definitely that a proper balance is required for initiation of the reproductive state, though it has been found on several occasions that a relatively high carbohydrate content exists in already flowering or fruiting plants. When analyses have been made of plants during the approximate period of flower inception, *a relatively high nitrogen content has been noted to be associated with flower initiation* (Knodel 22, Murneek 49, Asami and Ito 1).

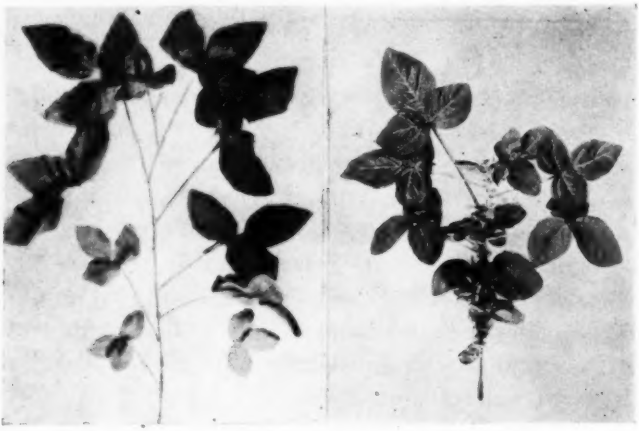

Fig. 1. Development of soybean plants, var. Biloxi, (left) under long photoperiod—vegetative, (right) short photoperiod—reproductive.

In many plants, particularly biennials and perennials, usually a considerable and often a long period elapses between the inception of floral primordia and the visible appearance of flower buds or flowers. During this time several factors, both external to the plant (soil nutrients, photoperiod, temperature, etc.), and internally (correlation of parts, hormone secretion, etc.), may so change the development or alter its food reserves (carbohydrates and nitrogen compounds, for instance) that no relationship can be made between chemical composition and initiation of the reproductive state. An illustration may be drawn from the influence of the photoperiod, which is a most effective means of modifying the development of many plants (Garner and Allard, 10), to reproduction of the soybean, var. Biloxi (Fig. 1).

In analyzing the effects of relatively short photoperiods to flower induction of this legume and the concomitant changes in carbohydrates and nitrogen composition, it was found (Murneek and Gomez 47, and Murneek 49) that under a short (7 hour) photoperiod the formation of floral primordia took place during the first 4 to 6 days, counting from the day the plants appeared above ground. Probably a few more days are required to completely induce maximal flowering in these young seedlings. During this general period the food reserves were rather meager and there was a *lower* relative carbohydrate-nitrogen concentration in the stems and elsewhere than during subsequent development of the plant (Murneek 49) (Fig. 2 and Table 1). It is apparent, there-

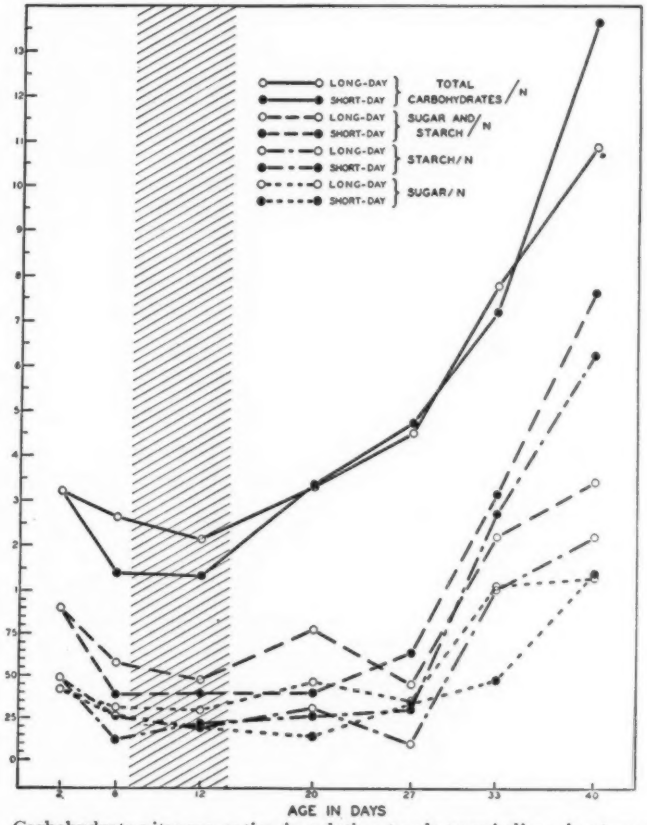

Fig. 2. Carbohydrate-nitrogen ratios in relation to photoperiodism, in stem of soybean plants, var. Biloxi. Short-day plants reproductive. Maximal flower induction period shaded.

TABLE I

CARBOHYDRATE-NITROGEN RELATIONS (RATIOS) IN STEMS OF SOYBEAN PLANTS, VAR. BILOXI, WHEN GROWN UNDER CERTAIN PHOTOPERIODS

Description of Plant Material	Sugar / N	Starch / N	Sugar and Starch / N	Total C-h* / N
Plants 2 days old				
Before exposure to photoperiod. Whole stem	.42	.48	.90	3.25
Plants 6 days old (2 + 4 days)				
Long-day, height 10 cm. Whole stem.....	.31	.26	.57	2.61
Short-day, height 10 cm. Whole stem.....	.26	.12	.38	1.40
(Beginning of induction of reproduction.)				
Plants 12 days old (2 + 10 days)				
Long-day, height 17 cm. Whole stem.....	.29	.28	.47	2.15
Short-day, height 17 cm. Whole stem.....	.18	.21	.39	1.36
(Induction probably completed.)				
Plants 20 days old (2 + 18 days)				
Long-day, height 24 cm. Whole stem.....	.46	.31	.77	3.33
Short-day, height 20 cm. Whole stem.....	.14	.26	.40	3.34
(Primordia formed, but no visible flower buds. Growth inhibited.)				
Plants 27 days old (2 + 25 days)				
Long-day, height 32 cm. Whole stem.....	.36	.10	.45	4.50
Short-day, height 21 cm. Whole stem.....	.34	.30	.64	4.68
(Flower buds distinct.)				
Plants 33 days old (2 + 31 days)				
Long-day, height 41 cm. Whole stem.....	.17	1.07	2.24	7.72
Short-day, height 23 cm. Whole stem.....	.47	2.72	3.17	7.22
(Buds, flowers and small pods.)				
Plants 40 days old (2 + 38 days)				
Long-day, height 47 cm. Whole stem.....	1.27	2.20	3.47	10.82
Short-day, height 24 cm. Whole stem.....	1.35	6.25	7.60	13.60
(Buds, flowers and pods up to 4 cm.)				

*C-h refers to total carbohydrates or the sum of total sugars, starch and hemicellulose.

fore, that *the initiation of the reproductive state can not be connected in cause and effect relationship with a relatively high, or any other, C/N ratio.* Since then it has been noted that when older vegetative plants are switched to a photoperiod conductive to reproduction the initiation of floral primordia may take place within 2 or 3 days (Borthwick and Parker 3, and Hamner and Bonner 16). This would appear to exclude the possibility of accumulation of reserve carbohydrates and nitrogen substances.

Additional evidence may be presented from changes in chemical composition of stems (where induction and development of reproductive organs occurs) of soybean plants that have been switched over from long to short and short to long photoperiods (Table 2). When flower initiation took place (two lower groups of Table 2) the carbohydrate-nitrogen ratios were relatively low in comparison to vegetative plants (two top groups). Moreover, no significant relationship could be established between forms of nitrogen substances and those of carbohydrates, as has been suggested by some investigators (Nightingale 54)

TABLE II

"SWITCHOVER" EFFECTS ON NITROGEN AND CARBOHYDRATE METABOLISM OF SOYBEAN PLANTS, VAR. BILOXI.

Description of Plant Material	(Percentages on Dry Weight Basis)												
	Total N	Coagulable N	Ammonia N	Amid N	α-Amino N	Nitrate N	Sugars	Starch	Hemicellulose	Total C-h	Starch / N	Sugars and Starch / N	C-h / N
Long-day plants — No flower induction													
(18 days old)													
Height 22 cm.													
Upper stem	3.55	1.196	.038	.457	.709	.531	2.86	2.29	9.35	14.50	.65	1.17	4.08
Lower stem	2.16	.496	.023	.277	.412	.575	3.18	.59	9.76	13.53	.27	1.74	6.26
Short-to-long — Induction, but no flowers													
(9 + 9 days old)													
Height 20 cm.													
Upper stem	3.80	1.182	.029	.442	.797	.509	3.26	1.18	7.23	11.67	.31	1.15	3.07
Lower stem	2.60	.571	.023	.385	.614	.611	3.18	.81	10.79	14.78	.31	1.54	5.68
Short-day plants — Induction and flowers													
(18 days old)													
Height 17 cm.													
Upper stem	5.34	1.496	.022	.802	1.400	1.298	.88	.58	6.02	7.48	.11	.27	1.40
Lower stem	3.85	.797	.065	.524	.813	1.373	.69	.66	8.76	10.11	.17	.35	2.62
Long-to-short — Induction													
(9 + 9 days old)													
Height 19 cm.													
Upper stem	4.59	1.309	.029	.375	.992	1.152	.88	.44	5.90	7.22	.10	.29	1.57
Lower stem	2.43	.508	.033	.143	.477	.998	.58	.36	10.87	11.81	.15	.39	4.86

Then, too, there is much evidence on record that even drastic changes in nutrition of the plant can not destroy reproduction induced by appropriate photoperiods (Eaton 8, Neidle 53).

That observable chemical alterations occur in tips of stems, in leaves and probably elsewhere when plants are exposed to a photoperiod conductive to flowering is evidenced, for example, by changes in catalase activity, but such differences most likely are correlated with changes in growth response of the plant than with reproductive development (Hibbard 17) (Fig. 3). The fact should never be lost sight of that the photoperiod frequently affects just as quickly and strikingly the vegetative as the reproductive development of a sensitive plant and that normally initiation of reproduction is independent of rate of growth Almost all of the chemical changes so far observed in reproductive Biloxi soybeans are most likely due mainly to photoperiodic inhibition of growth and not necessarily parallel the induction or development of flowers, the two often overlapping, however.

Hormone theory of reproduction:

The current evidence points to the fact that initiation of reproduction is of a catalytic nature and that a hormone may be responsible for the induction of floral primordia. The idea that a specific hormone-like substance is produced in the leaves as a result of exposure of a plant to suitable photoperiod, temperature, etc., is gaining ground at present. The concept is based on the suggestion by Sachs (62, 63) that flower producing substances ("Blühstoffe") are formed in leaves and transported to certain terminal and axillary meristematic regions of the stem where flowers are initiated. This still hypothetical substance has been tentatively named "florigen" (Chajlachjan 6). In long-day plants it is synthesized only under a long photoperiod or in continuous light, in short-day plants under a short photoperiod, and in plants non-sensitive to photoperiod under all natural lengths of day. It may be diffused from one part of the plant to another up and down the stem (Chajlchjan 6, Moshkov 34, Hamner and Bonner 16), and even from the scion of one species through a graft union into the stock of another (Moshkov 34, Melchers 31, Kuijper and Wiersum 25). Florigen evidently is non-specific in character.

Experimental evidence shows that in some plants the production of the flower forming hormone depends also on temperature (Thompson

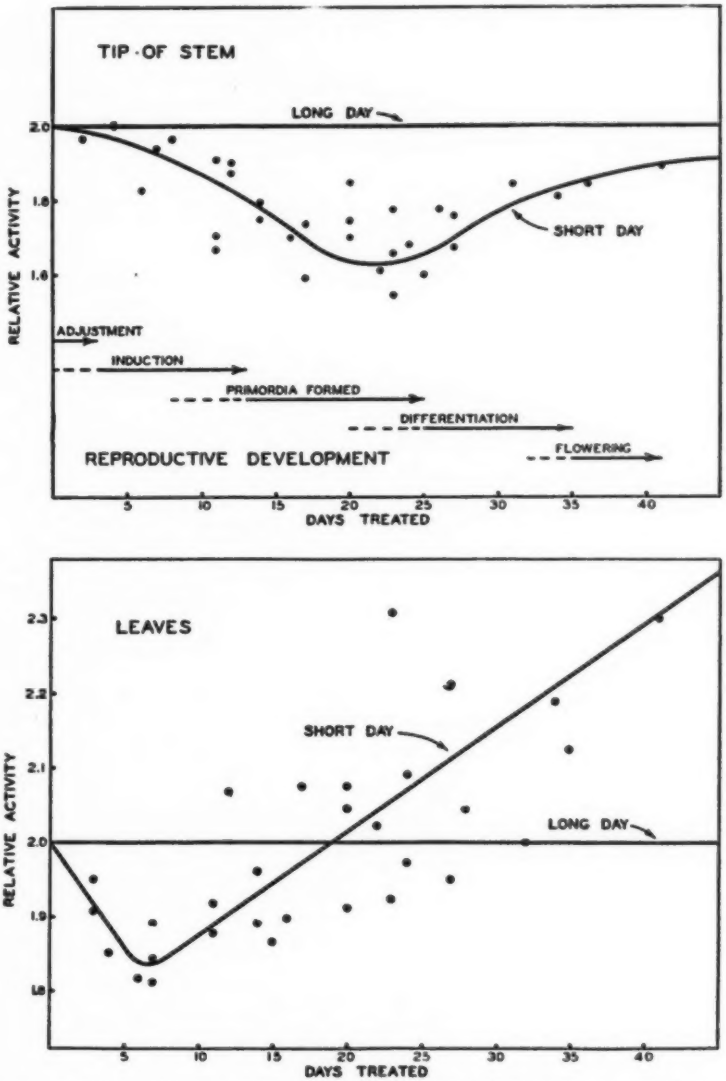

Fig. 3. Relative catalase activity in short-day Biloxi soybean plants, expressed as log percent of long-day ones.

66, Post 55). Sometimes it is difficult to know whether the effect has been due solely to temperature or in relationship with photoperiodism. In the latter case, temperature following photoperiodic induction may

Fig. 4. Relative growth in height of plants of *Rudbeckia bicolor*. Left—long-day reproductive plant. Right—short-day vegetative rosette.

only permit or hasten the development of the flowers, thus influencing or continuing the formative effects of the photoperiod (Gilbert 11, Eaton 8, Hurd-Karrer 19, Purvis 56).

When *Rudbeckia bicolor*, for example, is kept in a short day it will remain in a rosette stage almost indefinitely if the temperature is not abnormally high (Fig. 4). When, however, during the day it rises to 95° F. and higher, then these "vegetative plants" will form large flowers on the main stem (Fig. 5). In such instances it may be considered that high temperature initiated and permitted to develop flowers. Apparently, as regards sexual reproduction, this species of *Rudbeckia* is "attuned" to at least two environmental factors, a long day and high temperature. In this connection it was interesting for

Fig. 5. High temperature induces flowering in "vegetative" plants of *R. bicolor* but does not stimulate growth of main stem.

the writer to discover that a more northern species, *R. speciosa Newmanii* (Wendrath), while it is also a long-day plant, did not form flowers under high temperature in a short day. Is it adjusted in this respect to long days only, or to still some other environmental factor (s) in its more northern habitat?

In considering the relation of temperature to photoperiod as factors in sexual reproduction of plants, the phenomenon known as vernalization should be considered. When slightly moistened seeds are maintained for a period at certain temperatures, depending on the species, there is a physiological induction of reproduction, though, of course, no visible floral primordia are formed in this extremely early, still embryonic stage of the life of the plant. The significant feature is that such vernalized seeds may be dried after completion of the treatment without loss of induction, though "devernalization" may be demonstrated under certain circumstances. When vernalized seeds are sown subsequently, flower and seed development takes place much sooner than normally, as for example winter wheat flowering in the same year. In most cases, though, this will happen only if vernalization (temperature induction) is followed by a proper photoperiod during the early growth and development of the plant.

According to Lysenko (28, 29), who has developed techniques and made attempts to analyse the phenomenon, many plants have to pass through two, possibly more, stages or phases of internal readjustment, the thermo- and photo-phase, at least, before they can become sexually reproductive. There are others in which the two phases may be completed more or less concurrently. Surprisingly enough, induction, either thermo or photo or both, may occur while the seed is developing and maturing on the mother sporophyte, even as unbelievably early as the 8-cell stage (Gregory and Purvis 13, Zarubailo 73). The concept of "phasic development" of plants has been discussed in detail elsewhere and therefore need not be elaborated here (Maximov 30, Whyte 72). Many aspects of the photoperiod and temperature in relation to adaptation of various species, varieties and ecotypes are being studied at present (Eguchi 9, Roberts and Struckmeyer 60, Knott 23).

Physiological induction during various stages of development of the seed and thermo induction after the seed has matured, while its mechanism is unknown, point unmistakably to a mormone-like action in the initiation of the sexual state. Some have gone even so far as to

postulate a special hormone, *vernalin,* as the active agent in vernalization (Melchers 32). Whether this hormone, if present, is the same as the postulated *florigen* is unknown.

There is no information as to the chemical nature of the flower initiating hormone. There may well be two hormones, one responsible for the formation of the male, the other the female reproductive organs (Chajlachjan 6, Savelli 64). It seems to be produced in very minute quantities and promptly in the mature leaves in a suitable environment and in proportion to the amount of foliage present. Opinions differ as to whether it is formed in light (photoperiod) or in darkness (nocturnal period.)

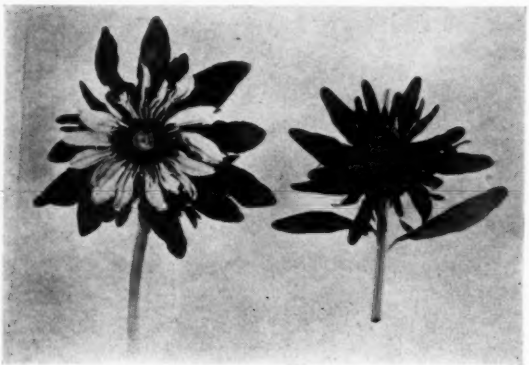

Fig. 6. "Vegetative" flowers of *Rudbeckia* due probably to partial induction of reproduction.

Moving to certain terminal meristems, whose positions are undoubtedly established genetically, the hormone in some way affects the apical cells, changing their activity from production of vegetative organs only to the inception of floral primordia. Meristems nearest to the "hormonizing" leaves probably get the first chance or largest dosage of the catalyst, though the transport to other regions seems to be quite rapid. The physiological state of "ripeness to flower" may involve the whole plant or may be localized to particular meristems, while others remain temporarily or permanently vegetative. In case a photoperiod is used as the primary environmental stimulant, a certain number of days seem to be required for the complete tissue activation by the hormone. Lacking this, inception of flowers may be localized to specific parts of the plant and in those only partially expressed (Biddulph 2, Murneek 46, 51) (Fig. 6).

There is a great difficulty in analyzing the action of the hormone in floral initiation and development, since any of the diverse substances through which the florigenic stimulus may express itself morphologically may become at times limiting factors. *Though physiologically induced, floral primordia may not be formed if the internal or external environment is unfavorable, and though in an appropriate environment reproduction may be initiated irrespective of the condition of food resources of the plant, it does not mean, however, that reproductive organs will always develop or function. Food reserves are of great importance in flower development but particularly so for the growth of seeds and fruits.* There is the possibility, also, that the development of floral organs may be promoted by other catalysts produced concomitantly in the plant. In this connection the discovery that acetylene induces prematurely flowering in the pineapple seems to be highly significant (Lewcock 26). Though much study is still required, some have hastened to express the belief that *florigen* fosters and regulates sexual reproduction all the way from inception of floral organs to seed development (Chajlachjan 6).

Chief aspects of flower production:

In general, one may recognize the following salient features of flower inception and development in higher plants:

1. Genetically determined loci or terminal meristems, where the hormone may be received and the condition of "ripeness to flower" established physiologically. It is possible that frequently there are more such meristematic points than there is available hormone. Some of them, therefore, may get but a limited amount—not enough to initiate floral primordia. Here we have then the first elimination of certain mersitems as potential flower producers.

2. With a relatively large number of meristems made "ripe of flower" and having formed floral primordia, it is very likely that many of them will be eliminated early because of lack of supply of building material. Organic nitrogen compounds most likely play an important role here, though a shortage of carbohydrates and other substances may at times also ensue, especially under extreme conditions of metabolism. Possibly other hormones necessary for the early development of floral organs should be considered also as factors.

3. Further elimination of a large proportion of the developing flowers

undoubtedly takes place because of competition for available food supply during their growth. *Far more floral primordia are usually formed than can possibly develop into functional flowers.* This can be demonstrated easily with many plants by disbudding and defloration experiments. When, for example, visible flower buds are removed repeatedly from a reproductive soybean or *Xanthium* plants, new flowers are produced repeatedly and continuously over a long period, till the total exceeds by far, often several times the number that the respective plants would normally form under the particular environmental conditions. (Murneek 51). During this time there is probably no further extension of tissues where flower buds may be produced anew, but possibly some additional differentiation of floral organs. The greater the limitations in soil nutrients, mainly nitrogen, or in supply of carbohydrates (extreme temperature or lack of light), the fewer flowers will be brought to completion (Murneek 36). Inhibition in flower development under adverse circumstances may take place at various stages of their development.

In the case of some dioecious (hemp) and monoecious (corn, cucumber) and possibly also hermaphroditic (tomato) plants, it has been noted that an increased nitrogen supply fosters an augmented production of female flowers, while carbohydrate deficiency suppresses formation or interferes with function of male flowers (Tiedjens 68, Tibeau 67, Howlett 18, Sabinin 61). For further information the reader is referred to a very complete review of the physiological aspects of sex development in angiosperms by Loehwing (27).

4. Not all flowers that come to anthesis are able to function normally, i. e., to form gametes and participate successfully in fertilization. The reasons may be very diverse. Some flowers, though appearing normal on casual observation, are different in many essential morphological structures, such as incomplete development of stamens or ovaries or the micro—or macrogametophytes. In other instances the gametic formation and union may be irregular for genetic or other reasons. In many cases of abnormal structure or function of flowers the causal factors undoubtedly are physiological. It is a common knowledge that plants very often produce an enormous number of flowers of which only a small proportion participate in fertilization. The reason for this is not always due to fundamental genetical or immediate external environmental causes.

Attention should be called here to an observation which may be of vital importance in consideration of the physiology of sex in plants (Murneek 49). It was found that when plants have become reproductive through exposure to an appropriate photoperiod, there is an increase in carotene and xanthophyll content of the leaves, as the following table shows:

TABLE III
CONCENTRATION OF CAROTENOIDS IN VEGETATIVE AND REPRODUCTIVE PLANTS AT APPROXIMATE TIME OF ANTHESIS

	Milligrams in 10-gram sample of leaves	
	Carotene	Xanthophyll
Cosmos		
Vegetative (long day)	0.95	1.5
Reproductive (short day)	1.17	1.85
Salvia		
Vegetative (long day)	1.85	2.50
Reproductive (short day)	2.07	2.80
Soja		
Vegetative (long day)	1.10	1.52
Reproductive (short day)	1.49	2.00

The concentration of these carotenoids seems to reach a maximum at the time of flowering, after which there is a reduction (Table 4). The lower amounts of carotene and xanthophyll in the nodes of soybean plants, var. Biloxi, when fruit is present suggests that either the carotenoids are moved to the reproductive organs or changed into some other compounds, possibly of vitamin A like nature.

TABLE IV
CAROTENE AND XANTHOPHYLL IN LEAVES OF SOYBEANS

	Milligrams in 10-gram sample
Vegetative (long day) plants	
Carotene	0.32 → 0.46 → 0.65 → 0.76
Xanthophyll	1.1 → 1.96 → 1.57 → 1.25
Reproductive (short day) plants	*Flowering*
Carotene	0.35 → 0.43 → 1.26 → 1.07
Xanthophyll	1.2 → 2.1 → 2.44 → 1.77

While the relation of carotenoid pigments to sexual reproduction in animals is well known, there is supporting evidence relative to their increase during flowering (Virtanen and Hausen 71, Nagasima 52). Some have brought them in relation to sex expression, mainly in the lower forms of plants (Moewus 33, Chodat and Schopfer 7). Moewus

has found that crocin, a water soluble carotenoid, will induce motility (sexuality) in *Clamydomonas* in as weak a concentration as 1: 250,000,000,000,000 parts of water and that 3 parts of cis-crocetin-dimethyl ester plus 1 part of trans-crocetin-dimethyl ester will form female cells and 1 part of the former to 3 parts of latter, male cells. If verified, this should be considered as a fundamental discovery.

Fertilization and formation of the zygote.

The most important feature of sexual reproduction of plants is fertilization and production of the embryo. Only in exceptional cases are seeds (apogamy) and fruits (parthenocarpy) produced without this process. If fertilization does not occur and the zygote is not formed, the fruit will grow but slightly and will usually absciss while still immature (Murneek 43, Tukey 70).

Immediately following fertilization there is a notable increase in metabolism of the ovule, ovary and accessory tissues. Without successful fertilization the nucellus and the integuments are subject to prompt atrophy (Murneek 51). In some species (*Citrus,* etc.) the presence of a true embryo often leads to the development of apogamic embryos from the nucellar tissue.

That fruit development is stimulated by the embryos is a well-known fact. Their size, chemical composition, and keeping qualities are controlled to a large extent by the number and position of the enclosed embryos (Murneek 45, 42, Tukey 69, 70, Reinecke 59). Parthenocarpic fruit have been produced recently by the application of auxin, heteroauxin, and other hormones, and pollen extracts to the style or ovaries, (Gustafson 14). A higher auxin content has been found in the ovules and seeds, which suggests that, in addition to the pollen, they probably produce it also and supply to the fruit (Gustafson 15). In most instances, parthenocarpically formed fruit are different in size and form in comparison to those containing seeds.

Not only the tissues forming fruit are stimulated as a result of fertilization, but the effect seems to extend to the flower stem, twig, and, in some plants, probably throughout the organism. In the apple, for instance, flowering and fruit setting is typified by a marked increase in all active forms of carbohydrates and nitrogen in the fruit spur, whence they are translocated to the reproductive organs. After fertilization, the direction of movement of most organic compounds is to the

fruits that have set, where large quantities eventually accumulate (Murneek 40). In the tomato, the flowers and young fruits apparently influence the whole plant. Following fertilization and formation of the zygote, there is an increased absorption of soil nutrients and a greater production of carbohydrates. This effect can be measured only if the number of embryos per plant is relatively large and the fruit is removed in an early stage of development, thereby preventing a masking effect due to the subsequently large food requirements and an injurious drain by the fruit (Murneek 38). In the case of corn, it has been found that the greatest production per acre of dry matter, protein and other plant constituents took place when seed formation was ample (Brunson and Latshaw 5).

The beneficial effect from fertilization and growth of the zygote is received chiefly by the tissues that become the fruit, the rest of the mother sporophyte receiving but a transitory stimulation as it were. In the presence of a relatively large number of embryos under the existing conditions of nutrition, often all of the synthesized and most of the currently absorbed nutrient substances go to the developing seeds and fruits. In certain types of plants the food requirements of the fruit are supplied from storage reserves though most of it probably is currently synthesized by the leaves. Hence a proportionally large leaf area is conducive to an increase in size of many fruits (Murneek 41). Similarly, ringing of branches of trees will frequently lead to an augmented fruit set and size, due probably to a temporary damming up of synthesized food substances (Greene 12, Murneek 48).

Influence of seeds and fruit on the mother plant.

Because of the draft of the available food supply by the developing embryos and accessory tissues, all sorts of inhibitions, modifications and even destruction of certain parts of the parent organism may take place, if the food resources are not ample and the demand is relatively large (Murneek 36). The tomato may be used as an illustration. When it carries a relatively large crop of fruits, the results will be retarded vegetative development, particularly when the nitrogen supply is restricted (Fig. 7). With extremely low amounts of nitrogen present in the plant and none coming from an external source, further growth of the stem is inhibited even by a single fruit. The terminal growing point is finally obliterated, the fruit becoming the main developing

Fig. 7. Plant to the right had two clusters of young fruit removed. To the left is a normal fruiting plant. Note difference in growth.

organ. A timely removal of it, however, results in recovery of vegetativeness and subsequent renewal of growth of the stem. On this new terminal extension flowers are formed once more and, when fertilized, may set fruit (Fig. 8).

The inhibitory effects of the seeds and fruits follow approximately in the following sequence: (a) Reduction in fecundity of the flowers, (b) decrease in number and size of flowers, (c) abortion of flower buds, (d) cessation of elongation of the stem, and (e) exhaustion of the whole plant, which under extreme conditions may result in death of all parts save the fruit (Murneek 36, 42). Detailed morphological and histological changes undoubtedly accompany the correlative influence of

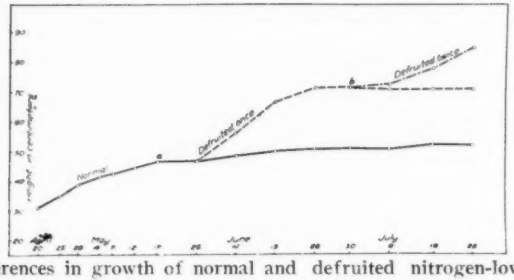

Fig. 8. Differences in growth of normal and defruited nitrogen-low tomato plants. First defruiting at *a*, second at *b*.

flowering and fruiting on the vegetative parts of a plant. (Psarev 58, Struckmeyer and Roberts 65).

A very favorable material for the study of the effects of fruit and seed development on inhibition of other parts or organs of the plant is the spider flower (*Cleome spinosa*). This robust annual produces fruits intermittently on long peduncles. The rapidly growing seeds monopolize the organic food supply (possibly nitrogen) to the detriment of flowers situated above them, with the result that the pistils become nonfunctional and often abort. By a timely removel of the pistils, in which fertilization had taken place but the zygotes had developed for only a few days, all flowers are formed normally and are fertile (Murneek 39, 35).

Fig. 9. *Corypha umbraculifera,* flowering (left), after fruiting (right). Photo by Skene, Ceylon.

Perennial plants, especially the lignified ones, present somewhat more difficult cases for analysis. Usually they have comparatively large amounts of storage food reserves and conspicuous independence of various parts. But they, too, are at times greatly disturbed by excessive sexual reproduction, specifically by the development of seeds and fruits (Murneek 37). After an unusually heavy fruit crop trees often do not flower or set fruit meagerly the following season. Sometimes several years may be required for a complete recovery from abnormal fecundity. This cyclic flowering and fruiting of many perennials is a well known phenomenon. Some of them die promptly after sexual reproduction, whether this happens after a few or several years of vegetative growth. (Fig. 9).

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NUTRITIVE FACTORS IN PLANT GROWTH AND REPRODUCTION

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It is elementary to say that growth depends upon an adequate supply of available carbon, of nitrogen, of essential minerals and of water. The importance of these basic nutritive materials for plant growth (and reproduction) and the processes concerned in their metabolism have received a great deal of attention; in fact the relation of mineral nutrition, of photosynthesis, of nitrogen metabolism and of water to plant growth and reproduction has been a major subject of investigation for many years. Much of this research has been based on the assumption that growth of plants is limited by the supply of available carbon, nitrogen, essential minerals or water and that reproduction also is determined by the absolute amounts of these fundamental nutritive materials or their relative proportions. In many instances this assumption is correct and examples could readily be cited in which growth is materially affected by the ability of the plant to synthesize carbohydrates, by a deficiency of phosphorus, potassium or some other essential mineral element, by a lack of nitrogen or water. When all the nutritive factors enumerated above are supplied in the presence of adequate oxygen, optimum temperature and other favorable environmental factors it is usually assumed that the growth of a plant proceeds at its maximum.

It has been recognized for many years in animal nutrition that such a concept of growth is incomplete; that even though food, water, oxygen and minerals are furnished in adequate amounts growth and reproduction may be limited by an inadequacy of minute amounts of accessory growth substances, for example, the vitamins.

It was known that plants synthesize the vitamins and are the primary source of these substances for the animal and this very fact inclined physiologists to regard with scepticism evidence and suggestions that substances identical with or analogous to the vitamins may be limiting factors in plant growth. In fact, although the idea that growth

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substances play a role in the pysiology of the plant is not new, indisputable evidence of the validity of the hypothesis is recent and has resulted from the availability for investigation of some of the vitamins in chemically pure form.

It is now clear that minute amounts of specific organic compounds in some instances identical with vitamins are as necessary for the growth of plants as they are for the development of animals and that in many instances the growth (and reproduction) of a plant is materially affected by the amount of one or more growth substances available to the organism. The demonstration of the importance of these substances for plants materially alters our ideas on the controlling factors which may be concerned in plant development and modifies our concept of plant growth.

It is not possible in a paper of this character to discuss in detail the specific contributions which have been made to this subject. My own work begun at the Alabama Polytechnic Institute more than twenty years ago, carried on at the University of Missouri and continued at the New York Botanical Garden has been concerned chiefly with the growth of various microorganisms and of the excised roots of higher plants. On the basis of the results from these investigations and the important contributions of Schopfer, Bonner, Knight, Kögl, Sinclair, Leonian and others the following generalities seem permissible, subject of course to revision in the light of further evidence.

The growth of plants depends in part upon the presence of minute amounts of specific organic substances which are as important as are the carbohydrates, oxygen, water, nitrogen or the essential mineral elements. The number of growth substances concerned in the development of a plant is unknown. It is probably large; twelve or more chemically identified growth substances are now known including among others thiamin (vitamin B_1), vitamin B_6, nicotinic amide, pantothenic acid, riboflavin, biotin, ascorbic acid, pimelic acid and there is evidence for many others. Some growth substances function as coenzymes; they are not, however, growth catalysts since they are destroyed in the reactions in which they function, although but minute quantities are involved and the ratio between amount of growth substance and resulting growth is high. Many of the growth substances, perhaps most of them, are important for both plants and animals; that

is, they are concerned in fundamental metabolic processes common to all or almost all organisms. In the deficiency of a particular growth substance the metabolic process in which it functions is interfered with or stopped and as a consequence the development of the organism is slowed, modified or stopped. For example, in a deficiency of thiamin the metabolism of carbohydrates beyond the stage of pyruvic acid does not occur.

Some plants synthesize from elementary substances such as sugar, nitrogen, and minerals adequate amounts of all the growth substances needed for their development but others lack the ability to construct sufficient amounts of one or more and must be supplied the deficiencies from without. In nature the growth substance deficiencies of one organism are supplied by the excess synthesized by others. In some instances the power to synthesize a specific growth substance is entirely lacking, in others the plant under suitable conditions makes enough for slow growth; in other words, some plants are autotrophic for essential growth substances; others are heterotrophic to various degrees. Growth substance deficiencies vary with the organism; there is no master growth substance with which all organisms heterotrophic for growth substances must be supplied. For some, it may be thiamin; for others pantothenic acid, inositol, biotin, nicotinic acid or vitamin B_6 and still others may be unable to synthesize enough of several. In fact differences in the requirement for growth substances exist in some instances between closely related organisms, even between varieties in the same species. Furthermore, the production of a growth substance may be affected by the conditions to which the plant is exposed so that under one set it requires for growth a supply from without and under another grows satisfactorily with no supplement. When deficiencies of more than one growth substance for a particular plant are involved the principle of limiting factors applies; until the one most deficient is supplied the others are ineffective.

It appears further that growth substances are involved in reproduction and in some instances at least the ones concerned are those important for vegetative growth as well. This does not exclude the possibility that there are specific substances concerned in plant reproduction which are of little importance in vegetable growth. In fact some of the latter class have already been reported.

The discovery of the importance of growth substances for plants and the rapidly accumulating evidence of the considerable number of plants which are heterotrophic to a greater or less degree for one or more growth substances emphasize that in the future studies of plant growth dare not be limited to the supply of foods, minerals, water and oxygen but must include a consideration of the supply of growth substances.

The solution of such puzzling problems as the growth effects involved in hybrid vigor and pilyploidy, the mutual relations between organisms, the reason for the importance of media of natural origin, problems of reproduction and many others may lie in the field of growth substances.

THE EXPERIMENTAL ALTERATION OF HEREDITY*

(Abstract)

By

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Xray treatment of the chromosome results not only in mutation but in various chromosomal derangements including deficiencies, interchanges, inversions, and intercalations. Whenever a terminal segment is moved to another chromosome, it moves to another terminal position. Moreover, a segment is never added to the end of a chromosome; it always takes a position where another segment has been removed. All of these types of chromosomal derangement, and absence of the possible types which do not occur, may be accounted for as a result of simple breakage of the chromosomes, if it be assumed that the fragments possess the power of reattachment at their broken ends.

The genetic alterations induced by Xrays may be classified in three groups—mutations, deficiencies, and rearrangements. Another type of alteration, which may be independent of these three classes, is the so-called "dominant lethal"—that is the abortion of the embryo induced by the irradiation of male germ cells. Since the individual affected is lost before there is any possibility of genetic or cytological analysis, we can not determine the cause of the abortion. It may be due to lethal mutation or deficiency, but it may also be due to any other change in the male germ cell which prevents the development of the embryo.

The four types of genetic change listed—mutation, deficiency, translocation, and embryo abortion—are found together whenever the germ cells are treated with Xrays. It is possible that they are necessarily associated, that is, that all are the result of some single basic change produced by the treatment. But it is also possible that the four effects are independent in origin, and that they are always associated under Xray treatment only because we are here dealing with a very powerful agent which is able to affect all of these diverse processes. If the former is true we may predict that somewhere in the spectrum, between the Xray region which is genetically effective and the visible light region

*The national officers of THE SOCIETY OF THE SIGMA XI plan to publish this lecture in full, therefore only an abstract of the lecture appears here.

which is genetically ineffective, a point may be found at which genetic effect ceases—and all of these effects will stop at once. If, on the contrary, the associated effects are due to independent processes, all of which are affected by Xray treatment, it is possible that their spectral limits may be different. In this case, a point may be found where some of the processes may be affected while others are not. This would permit a more or less selective alteration of the germ cell and might make possible the production of mutations without the accompanying chromosomal derangements.

Experiments with ultra-violet radiation show that, by the use of appropriate wave lengths, embryo abortion, endosperm deficiency, and mutations may be induced without appreciable effect upon the frequency of translocation. This shows that the induced mutations are not merely effects of changed positions of the genes, as has been suggested on the basis of the Xray results. By the use of other wave lengths, deficiency and mutation may be induced without appreciable effect upon the frequency of embryo abortion. This suggests that induced embryo abortion may be the result of some change in the irradiated germ cell dependent upon absorption by a substance distinct from that involved in chromosome breakage and mutation.

The effects of different wave lengths upon the frequency of deficiency varies widely, and this curve of relative genetic effectiveness is not very different from the curve of relative absorption in nucleic acid.

HORMONIC INTERRELATIONS BETWEEN REPRODUCTION, MAMMARY GLAND GROWTH AND LACTATION*

By

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The story of the regulation of reproduction and lactation centers about the pituitary—a tiny endocrine gland located in the center of the head in a bony cavity at the base of the brain. It secretes a number of hormones which stimulate and control the activity of many other endocrine glands including the gonads, the mammary gland, the thyroid and parathyroid, the adrenal cortex and possibly the pancreas. Some of these glands in turn secrete hormones which play important roles in the adjustment of the organism to its external and internal environment. In addition, there is increasing evidence of pituitary hormones which play roles in the regulation of the metabolism of carbohydrate, fat and protein.

The pituitary has been described as the "brain" of the endocrine system. It might also be likened to the governor on an engine, because its activity is controlled in part by the hormones produced by the glands which it stimulates. The interrelation between the rate of secretion of the pituitary hormones and the glands stimulated maintains the cyclic condition in some cases, such as the ovaries, and the normal secretion of others. When for some reason either the pituitary or the other endocrine glands become abnormal either in the direction of over or under secretion, the finely balanced regulation breaks down with serious consequences to the animal organism.

The relation of the pituitary gonadotropic hormones to the reproductive cycle has been described by Dr. Guthrie. It will be referred to here only to describe the influence of the ovarian hormones upon the growth of the mammary gland. Under the influence of the follicle stimulating hormone (FSH) the Graffian follicle of the ovary enlarges with fluid containing the estrogenic hormone. Estrogen has been shown to influence the tissues of the genital tract and to stimulate the growth of the duct system of the mammary gland. However, as

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will be described shortly, its mode of action on the mammary gland is not direct but rather through the pituitary.

The complete growth of the mammary gland, i e., the growth of the lobule-alveolar system, only occurs during the first half to two-thirds of pregnancy. At this time not only is estrogen being secreted (probably by the placental membranes) but progestin, the hormone of the corpus luteum. These two hormones have been shown in extensive experimental work to play a role in the growth of the alveolar system. (For the details, see review by Turner, 1939).

The Role of the Pituitary in Mammary Gland Growth

Until 1936 it was generally assumed that the ovarian hormones acted directly upon the tissues of the mammary gland. Upon the removal of the pituitary, the injection of estrogen and progestin was observed, in a series of experiments at this Station, to be ineffective in stimulating gland growth of the mouse, rat, guinea pig and rabbit. It was then shown that implants of pituitaries from animals injected with estrogen or pregnant animals would stimulate gland duct growth in such animal. Pituitaries from males or non-estrus females give little response. That the factor stimulating gland growth was not identical with the lactogenic hormone was indicated first by the lack of growth response with non-pregnant cattle pituitaries containing considerable amounts of the lactogenic factor and more recently by the separation of the factor free of the lactogenic hormone. To this new pituitary hormone, the name *mammogen* has been given.

In order to study the physiological and chemical properties of this new hormone entity, it was necessary to formulate an assay method. As estrogen stimulated the growth of the duct system, it was tentatively concluded that there might be one mammogenic factor which influenced duct growth and a second mammogenic factor, stimulated by estrogen and progestin, which would influence the growth of the lobule-alveolar system.

So far only the duct growth factor has been studied. An assay method has been developed (Fig. 1) and considerable study of the content of this factor in cattle pituitaries of various types has been made.

During pregnancy there is a rapid rise in the amount of mammogen in the pituitary, reaching a plateau with a continued high level of secretion until the latter stage of pregnancy when there is a rather

marked decline. Comparison of dairy and beef cattle indicate greater secretory activity of mammogen by the former. As dairy cattle usually have larger udders than beef cattle, these observations are taken to indicate that the rate of secretion of mammogen may in part control the extent of growth of the mammary glands. These observations are of especial significance because they may explain in part the mode of inheritance of udder size upon a physiological basis, i. e., an inherited difference in the ability of the pituitary to secrete the mammogenic hormones.

From a clinical point of view, the discovery of the mammogenic hormones may uncover a more direct approach to the study of the cause and control of mammary tumors and cancers. If estrogens play a role,

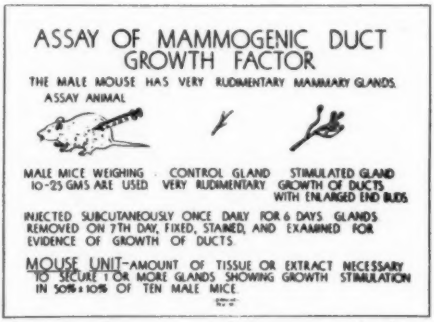
ASSAY OF MAMMOGENIC DUCT GROWTH FACTOR
THE MALE MOUSE HAS VERY RUDIMENTARY MAMMARY GLANDS.
ASSAY ANIMAL
MALE MICE WEIGHING 10-25 GMS ARE USED
CONTROL GLAND VERY RUDIMENTARY
STIMULATED GLAND GROWTH OF DUCTS WITH ENLARGED END BUDS
INJECTED SUBCUTANEOUSLY ONCE DAILY FOR 6 DAYS GLANDS REMOVED ON 7TH DAY, FIXED, STAINED, AND EXAMINED FOR EVIDENCE OF GROWTH OF DUCTS.
MOUSE UNIT-AMOUNT OF TISSUE OR EXTRACT NECESSARY TO SECURE 1 OR MORE GLANDS SHOWING GROWTH STIMULATION IN 50% ± 10% OF TEN MALE MICE.

Fig. 1. Technique for the assay of the mammogenic duct growth factor.

their action must be mediated by the pituitary resulting in the secretion of increased amounts of mammogen. What factors associated with maturity and old age other than pregnancy, and the ovarian hormones, might stir up the rate of secretion of mammogen and thus furnish the necessary stimulus for the unregulated growth of breast neoplasms?

As it is well known that the pituitary is quite responsive to stimuli both augmenting and suppressing the rate of secretion of its various hormones, a factor suppressing mammogen secretion might greatly reduce the rate of development of mammary tumors and cancers and thus control this disease.

The Pituitary Stimulates Milk Secretion. Not only does the pituitary stimulate the growth of the udder during pregnancy, but after calving, starts the milk secretion process. The hormone involved is called lactogen (galactin or prolactin). However, its function is not

only to start the process but the hormone is required for the maintenance of lactation as well.

Shortly after the discovery that the pituitary contained a true galactogogue, it was shown that the pigeon crop gland could be stimulated to growth or proliferation with pituitary extracts. In much of the work which followed, the pigeon has been used in the assay of this hormone (Fig. 2). Great progress has been made in chemically separating the lactogenic hormone from the other pituitary factors so that at the present time the lactogenic hormone is available in relatively pure form. (Claims for the preparation of a crystalline product have

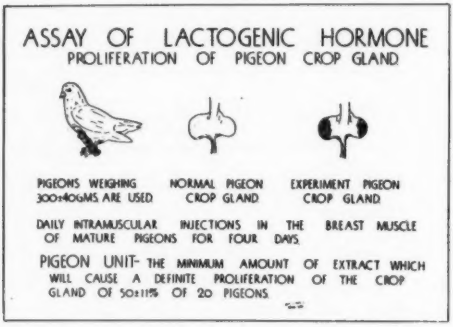
ASSAY OF LACTOGENIC HORMONE
PROLIFERATION OF PIGEON CROP GLAND
PIGEONS WEIGHING 300±40GMS. ARE USED
NORMAL PIGEON CROP GLAND
EXPERIMENT PIGEON CROP GLAND
DAILY INTRAMUSCULAR INJECTIONS IN THE BREAST MUSCLE OF MATURE PIGEONS FOR FOUR DAYS
PIGEON UNIT- THE MINIMUM AMOUNT OF EXTRACT WHICH WILL CAUSE A DEFINITE PROLIFERATION OF THE CROP GLAND OF 50±11% OF 20 PIGEONS

Fig. 2. Several methods are available for the assay of the lactogenic hormone. In our laboratory the pseudo-pregnant rabbit and pigeon are used. To induce good lactation in rabbits there is required about 260 pigeon units as defined above. A more sensitive pigeon test involving localized proliferation over one crop gland requires only one-twenty-fifth as much as indicated above.

not been confirmed). While at first a number of physiological functions were attributed to this hormone by certain investigators, the evidence is becoming increasingly convincing that the primary function is the stimulation of the mammary epithelial cells to milk secretion in mammals and crop gland proliferation in the pigeon and dove. In our laboratory the best lactogenic preparations as assayed in the pigeon are very effective in initiating lactation in the pseudo-pregnant rabbit.

In both laboratory animals and cattle it has been shown that there is a marked increase in the amount of lactogenic hormone in the pituitary following parturition. There is some evidence that the rate of secretion of this hormone then declines somewhat similar to the rate of decline with the advance of lactation, possibly explaining in part the mechanism controlling the persistency of lactation.

An interesting discovery has been made concerning the release mechanism from the pituitary. It has been observed that the stimulus of nursing or milking causes a discharge of the lactogenic hormone presumably to stimulate the formation of milk during the interval between milking.

That the lactation process requires a continuous supply of the lactogenic hormone has been demonstated by the operation in which the pituitaries of lactating animals have been removed. Milk secretion quickly subsides and only upon the regular injection of lactogen can milk secretion be reinitiated or maintained in such animals. Certain other pituitary hormones which have also been shown to be essential will be described later.

While these observations for the first time clarify the mechanism coordinating the phenomena of pregnancy, parturition and lactation, they have a wider significance to dairymen for they may explain some of the causes of differences in the productive ability of dairy cattle. Do some cows yield less than their maximum due to a lack of this hormone? In other words, would the injection of the lactogenic hormone to supplement the normal secretion cause increased milk secretion? A number of laboratories have reported definite increases in lactation in cattle and goats upon injecting the lactogenic hormone. These observations are taken to indicate that milk production in cattle may be limited by the rate of secretion of lactogen by the pituitary. While this method of discovering endocrine factors which may be limiting lactation will be of value in some cases, if two or more factors limit lactation, no apparent response would be expected. A more satisfactory method would be the direct determination of the rate of pituitary hormone secretion either in the blood or in the urine. While some lactogen has been recovered from urine, it appears to be present only in rather minute amounts. The blood deserves a more extensive study than has yet been devoted to its lactogen content.

Evidence of another type in favor of the idea that the rate of milk secretion is related to the ability of the pituitary to secrete large amounts of this hormone is appearing. Beef cattle as a rule have the capacity to secrete much less milk than dairy cattle. In a study in our laboratory it was observed that the pituitary glands from lactating and open (non-pregnant) dairy cattle contained 69 per cent more lactogen

per unit weight of anterior lobe tissue than did glands from lactating and open beef cattle; lactating and pregnant dairy cattle 73 per cent cent more; dry and open dairy cattle 72 per cent more and dry and pregnant dairy cattle 37 per cent more than the corresponding classes of beef cattle.

The Pituitary and the Adrenal. Upon the removal of the pituitary, it was found that the outer part of the adrenal called the cortex decreased markedly in size with cellular changes indicating reduced secretory activity. Later, extracts of the pituitary were observed to stimulate an increase in the thickness of the cortical zone indicating the participation of the pituitary in the physiological activity of the adrenal cortex. These observations were of especial interest to those studying lactation physiology due to the fact that when lactating experimental animals were deprived of their pituitaries surgically, lactation quickly declined and stopped. The injection of the lactogenic hormone alone was not able to maintain or reinitiate milk secretion. However, when the lactogenic and adrenotropic hormones of the pituitary were injected together, lactation was maintained if frequent injections of glucose were made to maintain the blood sugar level. That the adrenal cortical hormone played an indirect role in lactation was shown when lactation was maintained in hypophysectomized animals by the substitution of cortin for the adrenotropic hormone in experiments similar to those described above.

The function of cortin in relation to lactation is not clear. It is known that cortin plays a role in the regulation of the amount of salt present in the tissues and fluids of the body. If the secretion of cortin is low, salt is lost from the body in large amounts. At the same time there is a dehydration of the tissues associated with a large urinary secretion. Cortin thus may function in lactation chiefly in maintaining the water balance, permitting the flow of water and milk precursors to the mammary gland rather than withdrawing water from this gland. Cortin may also play a role in carbohydrate metabolism but not to the extent of maintaining the sugar level in lactating hypophysectomized animals.

Only indirect evidence for an increased rate of secretion of cortin in the lactating animal is available. A number of years ago it was shown that a life sustaining dose of cortin would not maintain lactation in

adrenalectomized rats. Later it was demonstrated that lactation could be maintained if several times the life sustaining dose were administered. It has also been shown that pseudo-pregnant rats, unlike similar rabbits, will not lactate upon the injection of lactogen. Recently, such rats have been induced to lactate when lactogen and cortin were administered together. These observations may be taken to indicate that increased cortical hormone secretion occurs during normal lactation and in the absence of such secretion milk yield may be limited or absent. Whether in normal lactating animals, the rate of cortin secretion limits milk flow, in some animals, will be studied as cheaper sources of the cortical hormone become available.

The Pituitary and the Thyroid Gland. The discovery of a pituitary factor which controls in part the activity of the thyroid gland marked an important advance in thyroid physiology. It was first observed that the thyroid showed signs of atrophy following hypophysectomy. Later crude extracts of the pituitary were found capable of restoring the normal thyroid cytology and also of stimulating an increase of size and degree of activity in the thyroid of some experimental animals notably the immature guinea pig.

The development of an assay of this pituitary factor, called the thyrotropic hormone, made possible the gradual purification of the active principle and the study of factors influencing the thyrotropic content of the pituitary. Until recently, the immature guinea pig has been widely used as an assay animal (Fig. 3). At the present time day old chicks are being substituted because of their low cost and availability.

Recent work in our laboratory has shown that the amount of thyrotropic hormone in the pituitary of the rat increases slightly during the latter half of pregnancy. Following parturition there was a further increase of about 40 per cent above the average pregnant level. Such an increase of thyrotropin should be reflected in a considerable increased activity of the thyroid gland and thyroxine secretion.

The character of fattening in beef cattle and milk production in dairy cattle have been points of selection for many years. Dairy cows which fatten readily instead of milking for a long period are discarded. The role of the pituitary in relation to these two characteristics is of interest. A study of the thyrotropic hormone content of beef and dairy

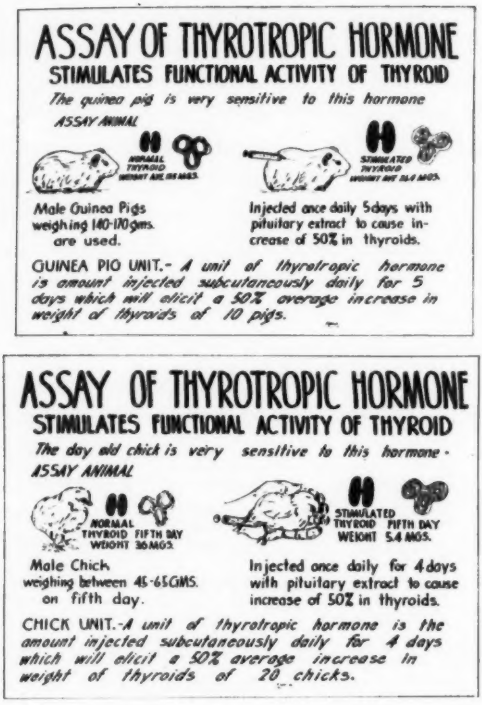
ASSAY OF THYROTROPIC HORMONE
STIMULATES FUNCTIONAL ACTIVITY OF THYROID
The guinea pig is very sensitive to this hormone
ASSAY ANIMAL
NORMAL THYROID WEIGHT ABT. 100 MGS.
STIMULATED THYROID WEIGHT ABT. 350 MGS.
Male Guinea Pigs weighing 140-170 gms. are used.
Injected once daily 5 days with pituitary extract to cause increase of 50% in thyroids.
GUINEA PIG UNIT.- A unit of thyrotropic hormone is amount injected subcutaneously daily for 5 days which will elicit a 50% average increase in weight of thyroids of 10 pigs.
ASSAY OF THYROTROPIC HORMONE
STIMULATES FUNCTIONAL ACTIVITY OF THYROID
The day old chick is very sensitive to this hormone.
ASSAY ANIMAL
NORMAL THYROID FIFTH DAY WEIGHT 3.0 MGS.
STIMULATED THYROID FIFTH DAY WEIGHT 5.4 MGS.
Male Chick weighing between 45-65 GMS. on fifth day.
Injected once daily for 4 days with pituitary extract to cause increase of 50% in thyroids.
CHICK UNIT.-A unit of thyrotropic hormone is the amount injected subcutaneously daily for 4 days which will elicit a 50% average increase in weight of thyroids of 20 chicks.

Fig. 3. For the assay of the thyrotropic hormone, immature male guinea pigs and day-old chicks are quite sensitive. As defined above, the male chick unit of thyrotropic hormone was found to be about 1/4 the amount of the guinea pig unit.

cattle pituitaries in our laboratory showed that those lactating non-pregnant dairy cattle contained 30 per cent more, lactating and pregnant 17 per cent more, and dry, non-pregnant 60 per cent more hormone than the corresponding classes of beef cattle. These results are suggestive of the influence of thyrotropin upon the capacity of dairy cows to transform their feed into milk rather than store it as body fat.

The function of the hormone of the thyroid gland, thyroxine, is generally considered to be that of regulation of general metabolism. When thyroid activity is low, body metabolism is low and there is a tendency to fatten; when the thyroid is over active, there is increased oxidation in the body cells, increased heart beat, and a thin condition of the body. Within the past few years it has been shown that thyroid activity influences the lactation process as well. Removal of the thyroid glands

of lactating cows and goats has caused a reduction of milk yield up to about 30 per cent. On the other hand, the feeding of dried thyroid tissue or the injection of thyroxine into dairy cattle especially during the declining phase of lactation has been shown to cause a marked rise in milk production as well as an increase in the percentage of fat. Cows differ in their response but increases of from 10 to 30 per cent in milk have been quite common. These increases have been interpreted as indicating a deficiency in the rate of thyroxine secretion by the animals due either to rate of secretion of thyrotropin or to a lack of capacity of the thyroid to respond.

Study of the iodine content of the thyroid gland indicates a storage of thyroxine during the summer months and an increased rate of secretion during the winter months. It has been explained by the fact that a cold environment stimulates increased secretion of thyrotropin and heat an opposite effect. May not these observations explain the reduced yield ofmilk during the warm summer months correlated with a reduced fat percentage?

The mode of action of thyroxine in stimulating increased milk and fat production is now under investigation. The increased heart rate indicates the probability that more blood flows through the udder in unit time. Whether the rate of secretory activity of the milk secreting cells is increased awaits further study. The composition of the milk precursors of the blodd might also be changed in such a way as to favor the more rapid uptake of these constituents. Thyroxine might also stimulate the rate of secretion of other endocrine glands such as the pituitary.

The Pituitary and the Parathyroid. The relation of the pituitary to the function of the parathyroid has not been studied extensively. There is some evidence that the parathyroids atrophy following hypophysectomy and further that pituitary extracts cause some parathyroid enlargement. The claim has been made that such extracts also increase the blood calcium.

The importance of calcium in milk secretion is well known. Lactating animals deprived of their parathyroid glands are subject to severe tetany unless the diet is fortified with calcium rich foods or vitamin D is added. Whether in normal animals, the yield of milk could be augmented by the addition of the active principle of the parathyroid is an open question.

The Pituitary and Carbohydrate Metabolism. Many years ago it was shown that blood glucose was used extensively by the active mammary gland. From 10 to 30 mgm. per cent are removed from the blood during each passage through the udder of the cow. Considerable evidence points to the metabolism of sugar as a frequent limiting factor in the yield of milk of dairy cattle. For this reason, the recent observations pointing to the control of carbohydrate metabolism by the pituitary has special significance.

In our laboratory pituitary extracts have been prepared which will stimulate a marked increase in the glucose content of the blood. In order to study the chemical properties of this hormone and methods of concentration, a bioassay method was developed using immature guinea

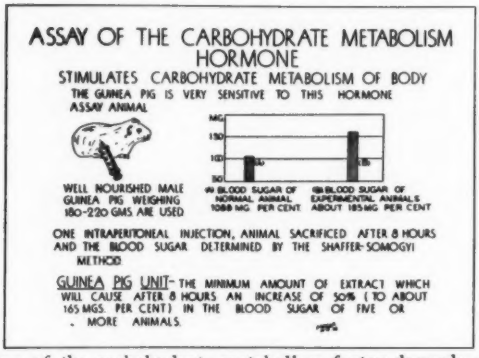
ASSAY OF THE CARBOHYDRATE METABOLISM HORMONE
STIMULATES CARBOHYDRATE METABOLISM OF BODY
THE GUINEA PIG IS VERY SENSITIVE TO THIS HORMONE
ASSAY ANIMAL
WELL NOURISHED MALE GUINEA PIG WEIGHING 180-220 GMS ARE USED
BLOOD SUGAR OF NORMAL ANIMAL 100 MG PER CENT
BLOOD SUGAR OF EXPERIMENTAL ANIMALS ABOUT 165 MG PER CENT
ONE INTRAPERITONEAL INJECTION, ANIMAL SACRIFICED AFTER 8 HOURS AND THE BLOOD SUGAR DETERMINED BY THE SHAFFER-SOMOGYI METHOD
GUINEA PIG UNIT- THE MINIMUM AMOUNT OF EXTRACT WHICH WILL CAUSE AFTER 8 HOURS AN INCREASE OF 50% (TO ABOUT 165 MGS. PER CENT) IN THE BLOOD SUGAR OF FIVE OR MORE ANIMALS.

Fig. 4. The assay of the carbohydrate metabolism factor depends upon an increase in blood sugar of immature guinea pigs.

pigs (Fig. 4). Certain investigators have suggested that the lactogenic hormone influenced the sugar level of the blood. Using this assay method it was possible to show that lactogen and "carbohydrate metabolism" factors are not identical for preparations rich in the lactogenic factor contained little of the carbohydrate factor while the best "carbohydrate" preparations contained little lactogenic hormone.

In hypophysectomized animals the blood sugar and lactic acid levels are significantly reduced. In rats the absorption of glucose is about 35 per cent below normal. The above observations explain the difficulty in maintaining lactation in hypophysectomized guinea pigs. Only by the frequent injection of glucose was hypoglycemic coma prevented in those animals which were maintained in lactation by means of lactogen

and the adrenal cortical or the adrenotropic hormones. It was suggested that the purified carbohydrate metabolism hormone would take the place of the glucose injections.

The Pituitary and Fat Metabolism. The large amount of fat secreted into milk calls for the mobilization by the lactating animal of huge quantities of fat either from the digestive system or from depot fat. Recent studies indicate that the blood lipids transformed by the udder into milk fat include neutral blood fat and possibly free fatty acids. Evidence for a relation between the pituitary and certain phases of fat metabolism have been appearing. It had been shown that crude extracts of the pituitary produced ketosis and an intense fatty infiltration of the liver. In our laboratory a study was made of the effect of

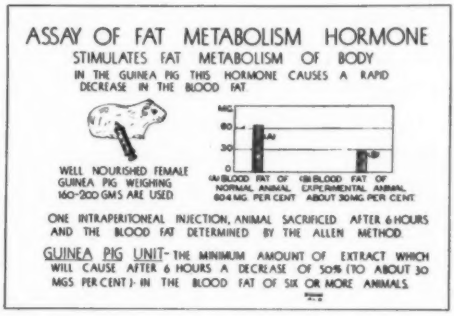
ASSAY OF FAT METABOLISM HORMONE
STIMULATES FAT METABOLISM OF BODY
IN THE GUINEA PIG THIS HORMONE CAUSES A RAPID DECREASE IN THE BLOOD FAT
WELL NOURISHED FEMALE GUINEA PIG WEIGHING 160-200 GMS ARE USED
MG.
60
30
0
(A) BLOOD FAT OF NORMAL ANIMAL 604 MG. PER CENT
(B) BLOOD FAT OF EXPERIMENTAL ANIMAL ABOUT 30 MG. PER CENT
ONE INTRAPERITONEAL INJECTION, ANIMAL SACRIFICED AFTER 6 HOURS AND THE BLOOD FAT DETERMINED BY THE ALLEN METHOD
GUINEA PIG UNIT- THE MINIMUM AMOUNT OF EXTRACT WHICH WILL CAUSE AFTER 6 HOURS A DECREASE OF 50% (TO ABOUT 30 MGS PER CENT) IN THE BLOOD FAT OF SIX OR MORE ANIMALS.

Fig. 5. The fat metabolism hormone of the pituitary rapidly depresses the lipids of the blood.

various pituitary extracts upon the blood lipids of the rabbit and guinea pig. It was shown that within a few hours after an injection, the blood lipids decreased very markedly when an initial extract of the pituitary was administered whereas the purified lactogenic hormone was without effect.

In order to further study the fat metabolism factor, an assay technique was developed using guinea pigs as assay animals as they are far more sensitive than the rabbit (Fig. 5). Preliminary study upon both the rat and rabbit pituitary indicates a content sufficient to make feasible a study of the variation in the amount of this hormone during maturity, pregnancy and lactation.

The Pituitary and Protein Metabolism. In many respects the factors

regulating the mobilization of nutrients for growth and for milk secretion by the animal body must be identical. In one case (growth) the nutrients are utilized by a wide variety of cells to make possible cell division resulting in growth of muscle and bone, in the other case (milk secretion) a localized structure, the mammary gland, takes similar nutrients from the blood and transforms them into milk in an ideal proportion for early growth of the new born. It seems reasonable that the metabolic problems confronting the animal organism in promoting rapid growth of the body may be closely allied to those promoting intense lactation.

If this were true the hormones affecting the two processes might have much in common. Thus it is believed that the role of the lactogenic hormone is primarily to stimulate the cells of the mammary gland to secretory activity possibly by activating the various synthetic enzymes involved. Lactation, however, can only continue in the presence of abundant milk precursors. It is believed that the mobilization of these milk precursors is dependent upon the pituitary factors regulating the metabolism of sugar, fat, protein and mineral matter.

Similarly, in the case of body growth, while there may be a factor which stimulates division of somatic cells (corresponding to the role of the lactogenic hormone), rapid growth is believed to be dependent upon the pituitary factors regulating the metabolism of the nutrients mentioned above, for only in their presence can body tissue multiply at a rapid rate. If this theory were true, a purified growth hormone would not stimulate the growth of hypophysectomized animals unless the metabolic hormones were supplied just as it was found that the lactogenic hormone was powerless to maintain lactation in the absence of other pituitary hormones.

At the present time there is no proof of a separate growth hormone in the sense postulated above, i. e., stimulator of somatic cell division. It is possible that the so-called "growth hormone" is a protein metabolism hormone and shows the characteristics observed due to the preeminent position of protein in the growth process. A number of studies have been reported indicating that the "growth hormone" markedly influences the efficiency of protein utilization and the storage of larger amounts of nitrogen in the body. These data are interpreted as indicating that the pituitary does secrete a factor which influences protein

metabolism and should be of importance not only in the growth process but in the secretion of milk protein as well.

The Inheritance of Milk Production. The preceding discussion is believed to give a picture of the amazing discoveries of the past few years answering for the first time the question of countless dairymen in regard to the cause of the marked differences in the size of the cows udder and their ability to secrete milk. Little doubt can remain that the endocrine system coordinated by the pituitary gland controls the processes regulating the growth of the mammary gland and the secretion of milk.

Cows inherit the ability to secrete varying amounts of milk but the endocrine studies have indicated the mode of physiological expression making possible these differences. The complexity of the problems involved in taking advantage of this gradually expanding body of knowledge in connection with the genetic improvement of dairy cattle are obvious. It will be necessary to develop methods of determining the rate of hormone production by the various endocrine glands which influence either the growth of the udder, the stimulus of milk secretion or the metabolism of the various precursors of milk in the blood Futher these methods must measure the rate of secretion of the hormones of the intact animal at a time of maximum physiological activity.

The most obvious plan would consist of measuring the level of hormone in the blood. However, the concentration of most hormones is rather low and unless very sensitive tests are developed minute differences could not be detected. There is also the possibility that the concentration in the blood might be maintained at a rather uniform level due to a threshold of excretion by the kidney. Further, the threshold level may vary in the presence or absence of other hormones.

The second type of test of hormone secretion rate would involve the measurement of the hormone concentration in the urine. Many hormones have been shown to be eliminated in a free or combined form in the urine. Here again there is need of sensitive assay methods. In both the urine and blood, progress has been made in extraction and assay of certain of the hormones of special significance in regard to the lactation process. However, much more must yet be done to correlate the amounts of hormone recovered with gland activity.

Indirect measures of endocrine gland activity may be developed. One good example is the response of certain lactating animals to thyroxine administration. May not this response indicate the lack of optimum secretion of thyroxine by the thyroid gland? Other hormones may be shown to have similar effect. This type of test has one serious defect namely the effect of the presence of two or more limiting factors. Thus if the rate of secretion of both thyroxine and cortin were limiting milk secretion there might be little or no response to the injection of either one alone.

While there are many apparent difficulties in the way of measuring endocrine gland activity, they are not believed to be insurmountable. Eventually it may be possible to determine the strength and weakness of families and individuals in regard to the rate of secretion of the various hormones which play a role in milk secretion. Such assays during periods of maximum physiological activity should determine the inheritance pattern comparable in a sense to the analysis of a soil for its content of various plant foods.

By the selection of animals which secrete the hormones in the most nearly proper proportions for maximum milk secretion or other productive purposes and mating animals which will supplement each others deficiencies, genetic science should begin to take a more dynamic role in the improvement of dairy cattle and other domestic live stock.

Note: For a complete review of the literature concerning the problems under discussion, the reader is referred to the author's chapter on the mammary glands in *Sex and Internal Secretions*, Edited by Edgar Allen, C. H. Danforth and E. A. Doisy. Pub. Williams and Wilkins, Baltimore. 1939, Second Edition.